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THE VITAMINES

BY

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College of Physicians and Surgeons

New York City

Authorized Translation
from
Second German Edition

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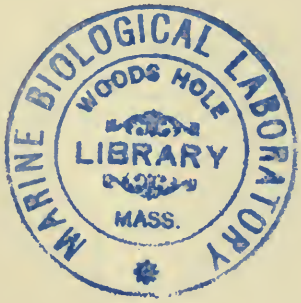

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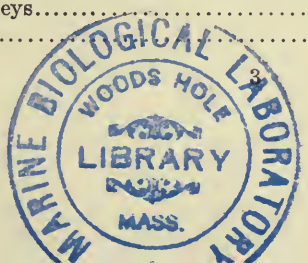

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PREFACE TO THE FIRST EDITION

This initial attempt at a classification of our knowledge on vitamines and avitaminoses contains, besides a number of facts, the personal conceptions of the author, new questions, ideas and lines of research. Much of the material is fragmentary while some of it is purely hypothetical. This small book is to be regarded as the first step in a new direction in the field of physiology and pathology. Many facts are still lacking in this field of work so that hypotheses must naturally fill the gaps. Even if the structure is skeleton-like, at least the fundamentals are well established.

At this point, I want to thank Dr. Fraser, Dr. Stanton, Prof. Holst, Dr. Mott, Dr. Roberts, Dr. Standwith and Dr. Zeller, and the London Society of Tropical Medicine, for the permission to reproduce illustrations. I am thankful also to Dr. Macauley of Cape Town for having called my attention to a South African cattle disease. In particular, do I wish to express my deepest gratitude to my Father, Dr. J. Funk, for his unvaried and tireless assistance.

I would appreciate it greatly if the investigators who are conducting research on problems discussed in this book would be kind enough to send me reprints of their work which, at times, is available only with great difficulty.

THE AUTHOR.

Cancer Hospital Research Institute,
Brompton, London, S. W.
October, 1913.

PREFACE TO THE SECOND EDITION

This edition, totally revised and almost completely re-written, appears seven years after the first edition, deferred because of the World War. In the interim, we have received many communications from our colleagues informing us that our effort had provided them with a welcome stimulus to their work. We have watched the great development of this field of research since the appearance of *The Vitamines* and it is a source of great pleasure that our work has also contributed to this progress. The views expressed at the time have been tested, for the most part, and found correct. While the first edition was published at a time when great differences of opinion prevailed, our desire then being to take cognizance of all of them, we feel justified now in reviewing only those investigations, the viewpoint of which is not too far removed from ours. In this way, it has been possible to make the book more comprehensive. The subject of vitamines, already beyond the stage of hypothesis, is based on a firm foundation and has received universal recognition. In spite of this, however, we are well aware that great gaps exist in our knowledge, so that we can not regard the chapter as closed.

These gaps may be explained by the fact that the field of vitamine research gave many investigators the opportunity of making a name for themselves with rather superficial work. Only seldom did they seriously endeavor to get at the basis of the phenomena observed. To permit of progress in the subject of vitamines, it would be very desirable that at least some of the workers should abandon the beaten path of exclusive animal experiments and break a fresh trail leading towards the chemistry, physiology and pharmacology of the vitamines, as well as the anatomical pathology of the avitaminoses.

For help in the preparation of this edition, we are indebted to many of our colleagues; we wish also to express our thanks to the Medical Research Committee (London) and to Dr. Alfred F. Hess (New York) for permission to reproduce a number of illustrations. In addition, we wish to thank those whose permission it was impossible to obtain because of uncontrollable circumstances. We are especially grateful to Dr. Richard Hamburger, First Assistant at the Pediatric Clinic, Berlin, who took it upon himself to correct and critically review the proofs.

In particular, however, we take pleasure in recording our appreciation of the assistance extended by the Hon. Herman A. Metz (New York) who, although a layman in this field of work, understood its significance and gave us the opportunity, after an enforced interruption of many years, of resuming the experimental work in the "Research Laboratory of H. A. Metz."

This book will be of interest not only to nutrition investigators and childrens' specialists, but to every physician who comes into touch with questions of the physiology of nutrition. It treats many problems which are closely allied to other lines of research and will be of value to plant physiologists, bacteriologists and animal breeders.

CASIMIR FUNK.

College of Physicians and Surgeons,
Columbia University,
New York City.
December, 1921.

TRANSLATOR'S NOTE

The translator has been associated with the author for three years in experimental vitamine research, and it is a pleasure to record at this point the spirit which animates him in his work and his earnest desire to see real progress made towards the solution of the many problems of nutrition. It was this which prompted the preparation of the present edition, intended rather as a stimulus to thought and further research than as an elaboration of technicalities. Inasmuch as the author has had the opportunity of reading the manuscript before it went to press, the reader may be sure that the sense of the book has been accurately preserved.

In conclusion, the undersigned wishes to extend his thanks to the Hon. Herman A. Metz for the many facilities provided in the preparation of this book.

Harry E. Dubin.

Research Laboratory of H. A. Metz,
New York City.
December, 1921.

INTRODUCTION

The first edition of this book, drafted seven years ago, was intended to serve the purpose of directing the attention of the medical and chemical professions to this new and attractive field of investigation. The findings upon which we based our views in 1913 appeared indeed to be far from indisputable but time has shown the comparative accuracy of our conception. Since then, we have made it a point to note any work that might have some bearing upon the question of the vitamines, and have now undertaken to point out what may be regarded as erroneous and what may be looked upon as correct, in the light of the present status of the subject.

Our original classification included beriberi, scurvy and Barlow's disease as true avitaminoses, while pellagra, sprue, rickets, and some metabolic diseases of animals, were held to be hypothetical avitaminoses. At present, beriberi, scurvy and Barlow's disease are universally accepted as true avitaminoses, while rickets is quite generally acknowledged as such. The etiology of pellagra and sprue, on the other hand, has not yet been definitely established. Regarding pellagra, which we shall discuss later, the dietetic hypothesis is at present in the foreground.

As for the chemistry of the vitamines, an examination of the literature reveals little progress in this direction. To be sure, one must bear in mind that the outbreak of the war was not particularly conducive to the long and tedious research that is necessary, if this phase of the subject is to be cleared up. From time to time, word comes from this or that laboratory to the effect that the puzzle has been solved—that progress has been made even towards the synthesis of the vitamines—but after much patient waiting, nothing more is heard of the discovery. However, since a large part of the experimental work is being carried on in many industrial research laboratories, it is quite possible that definite important progress has been made, of which reports have not yet been published.

Incidentally, the war has given added impetus to vitamine research in another direction. The vitamines have now become of great importance from the point of view not only of pure science and medicine, but also of political economy—much greater than could have

been foreseen when the first edition of this book appeared. The war, first of all, prevented the normal interchange of foodstuffs among the various countries. Subsequently, this was followed by a disturbance in the entire mode of living, due to the necessity of making war, the utilization of farm labor for other purposes, and the lack of means of transportation. Although, as this is being written, more than two years have elapsed since the end of the war, conditions have become worse instead of better, according to available reports. Similarly, notwithstanding the national rationing of food both during and after the war, the people have suffered because of the mistakes made during the war. In the face of higher prices, general unrest and unwillingness to settle down again to some productive work, the abnormal conditions already mentioned will likely prevail for several years to come. It is not our purpose to suggest that knowledge of the vitamines will solve the present difficulties. Still, we shall call attention to facts and principles which, at present, are of universal interest—principles which may contribute to the alleviation of the wretchedness of stricken Europe.

Considering more closely the dietaries of farmers and of the rural populations in various parts of the world, it is easy to see that no knowledge of vitamines is necessary to keep those people in good health. From generation to generation, their nutrition has been regulated according to the climate, the economic situation, and the exigencies of the work performed. Of course, in some oriental countries, where conditions are not so well known to us, instinct sometimes does not choose the correct food and hence the prevalence of beriberi. All in all, we see that the white races have a wholesome knowledge of their food requirements, which is only natural. In certain provinces, before the war, it was possible to see examples of particularly monotonous and simple dietaries accompanied, on the whole, by no pathological conditions. We may be sure, however, that since these same peoples apparently subsisted on a practically unchanging diet for hundreds of years, they would most certainly not have survived if their choice of diet had been anything but correct.

When the usual equilibrium is disturbed by extraneous conditions such as war, a financial crisis or a catastrophe, then the practical knowledge of centuries suddenly becomes useless to the people, and they are obliged to seek some other basis of existence. Untold hardships are endured till this is accomplished, the population func-

tioning similar to experimental animals, used to establish the value of new foodstuffs. Here, at least, there is the added advantage of successfully applying the various principles of nutrition and sharply terminating the unnecessarily protracted period. In this connection, it is not impossible to make immediate use of the enormous amount of information obtained on the subject, both before and during the war, especially by the United States and England. During the past few years, virtually all foodstuffs have been tested as to their nutritive value and their vitamine content. Although most of the data secured are the results of animal experiments, they are, with a few reservations, directly applicable to man—especially as it has been demonstrated that there are no vital differences between the findings obtained with man and those with animals.

In general, it may be said that during the war, and after, there was a disarrangement of the nutritional elements resulting in a decreased consumption of protein and fat and a greater intake of carbohydrate, together with a diminution of vitamines. It should be noted here that the more important dietary constituents, in comparison with the physiologically inferior ones, have increased greatly in price, so that there is this danger to contend with in addition to the element of scarcity. Now, when a well-planned animal experiment is undertaken, all factors are controlled as far as possible except that one whose influence upon the organism is being determined. Quite another state of affairs confronts us in the case of nutrition investigations on man, where conditions arise which are only rarely met with in animal experiments. If there is a lack of one constituent, then it is almost certain that the entire choice of diet is not correct. For example, if the protein content of the diet is too low, it is at the same time apparent that the carbohydrates are present in excess, that the vitamine content is diminished, and that the inorganic elements are inadequately grouped. It is easily possible that right at this point is where we must search for an explanation of the pathologically occurring avitaminoses, the etiology of which it is so difficult to establish. If in such a case as mentioned above, the missing factor in the diet is supplied, good results do not follow, for the reason that still other factors have not been taken into consideration.

Under certain circumstances, a seemingly well chosen diet may prove to be inadequate, particularly when special demands are made upon the individual, such as hard work, growth, birth, and nursing.

All these factors must be taken into account when commenting upon the pathological conditions which are of exceptional interest to us. These are chiefly the conditions which give us an insight into the causes, which are so difficult to determine in hunger edema and pellagra. Even a well informed physician may easily be led astray. He questions his patients about their diet, whereupon they enumerate a long list of foodstuffs, from which apparently nothing has been omitted. Immediately, his attention will be directed to obvious things, such as mode of living, nature of work performed, and method of preparing and cooking the food—everything which may be etiologically important and may help him solve his problem.

Despite the fact that a number of ideas originated by us are credited to others, it is a source of pleasure to witness the great progress that has been made in vitamine research. In our opinion, the name "Vitamine", proposed by us in 1912, contributed in no small measure to the dissemination of these ideas. The word, "Vitamine", served as a catchword which meant something even to the uninitiated, and it was not by mere accident that just at that time, research developed so markedly in this direction.

Our view as to the fortunate choice of this name is strengthened, on the one hand, because it has become popular (and a badly chosen catchword, like a folksong without feeling, can never become popular), and on the other, because of the untiring efforts of other workers to introduce a varied nomenclature, for example, "accessory food factors, food hormones, water-soluble B and fat-soluble A, nutramine, and auximone" (for plants). Some of these designations are certainly not better, while others are much worse than "Vitamine."

HISTORICAL SURVEY

In spite of the fact that the knowledge of the vitamines taken as a whole is not older than ten years, and although until lately. the idea was prevalent that for the complete nutrition of an animal organism only proteins, fats, carbohydrates, salts and water were necessary, there is nevertheless, in the older literature, no lack of statements which of themselves should have given rise to an eager search for additional dietary components essential to life. The progress which has already been made by research in vitamines removes all doubt as to the actual existence of such substances, and every year brings forth new findings which enhance the importance of the vitamines to life.

The scientific research leading to the conception of the vitamines proceeded through many intermediate stages, which we shall shortly describe. A great stimulus to the development of the modern science of nutrition was furnished by the investigations into the chemistry of the proteins which we owe, above all, to the classical work of Emil Fischer and Kossel. These investigations not only contributed to the knowledge of the composition of the proteins, but also gave rise to the study of the relationship between the individual dietary constituents. They demonstrated particularly, that the various proteins exhibited, qualitatively as well as quantitatively, a varied composition, and that naturally occurring proteins have not the same physiological value. Without going into details, which may be found in any text-book, we shall take up only those facts bearing upon the study of the vitamines. It has been shown by many investigations that certain amino acids, such as tyrosine, tryptophane, arginine and lysine, are more or less indispensable to the animal organism. This question is not yet completely settled, but we know, however, that some proteins, for example, zein (which is lacking in tryptophane), or gelatine (which lacks several important amino acids), are not sufficient for normal maintenance and growth. Latest developments also indicate that an animal will utilize a diet containing animal proteins, better than one made up of plant proteins. This conception is based preëminently upon the supposition that animal proteins have a composition more nearly related to body protein than do plant

proteins. That is to imply that the animal organism needs a smaller amount of animal protein to maintain its nitrogen balance. This view was utilized by Thomas (1) to group the various proteins of animal and plant origin according to their biological value. All these questions have exerted their influence upon the development of the study of the vitamines, and are even now closely related to many of our problems to which we shall call attention later.

Another matter that is of interest, is the extent of the ability of the animal body to synthesize many of the substances necessary for its existence. The peculiar deficiency symptoms, of which we speak in this book, have often been attributed to the apparent inability of animals to synthesize some of the body constituents. The substances which, of all others, come to mind here are the nucleins and the lipoids or phosphatides. If this were really the case, then these substances would also have to be regarded as vitamines, a conception which is upheld even now by some authors. The older animal experiments, conducted with purified food substances, showed that nucleins and lipoids actually could be synthesized. In spite of this, we encounter in the work of the last ten years, the observation that these substances have exhibited real therapeutic and nutritive qualities, an observation that found its chief exponent in H. Schaumann (2) who elaborated the theory of phosphorus insufficiency as an explanation of beriberi and similar diseases. This interpretation was vigorously opposed by the writer and gave him the chance to propound the theory of the vitamines. We shall speak of the nucleins and lipoids in greater detail in another chapter, but we shall also touch upon them lightly at this point.

Even before the vitamines were known, reports were published dealing with the synthesis of lipoids in the body. McCollum, Halpin and Drescher (3) working with chickens on a lipoid-free diet, showed that the eggs contained a normal amount of lecithin. Fingerling (4) and also Abderhalden (5) were able to show that animals could build up the needed organic phosphorus compounds from inorganic phosphates. Still, we find in the description of the condition of the above animals, some observations that point to the necessity of lipoids for life. It remained for vitamine research to clear up this matter completely.

In this connection, we shall mention, first of all, the important work of Stepp (6) in 1909. He made the significant observation that

mice cannot maintain themselves on a diet composed of bread, and milk which has been extracted with alcohol. If, however, the extracted portion is recombined with the diet, then the animals begin to grow once more. In another communication, Stepp (7) was convinced that his findings were not due to the loss of salts during extraction, and classified in the lipoid group, those substances essential to life. In spite of the fact that this conclusion was not quite justified, the work of Stepp really merited more attention than it received at the time of its publication. Unfortunately, this was not the case, for the relegation of the substances necessary for life to the class of lipoids, which already had been considered essential, did not signify real progress.

Aside from the work of Stepp, attention could easily have been focused upon the conception of the vitamines by research which had for its purpose the study of the importance of salts in nutrition. Relative to this, the school of Bunge is credited with a great deal of merit. Another series of investigations dealt with the utilization of purified foodstuffs, especially in the mouse, the rat and the dog.

In 1873, Forster (8) tried to determine whether or not dogs could maintain themselves on an ash-free diet. For this purpose, he fed the meat remaining after the preparation of Liebig's meat extract. These residues were washed repeatedly with distilled water until they contained only 0.8 per cent ash. They were then combined with fat, sugar and starch, and fed to dogs, with the result that they died sooner than starving animals. Experiments with pigeons, fed with casein and starch (and occasionally a little fat), gave the same results. The symptoms noted by Forster, in pigeons, were particularly interesting. They refused food, lost a great deal of weight, showed weakness, opisthotonos and characteristic circular motions. As we shall see later, these symptoms, which Forster attributed to a lack of salts, were apparently identical with "polyneuritis gallinarum," a disease of chickens described by Eijkman in 1897. This view is further strengthened when we note the length of time that the pigeons lived—13, 26 and 31 days. In dogs, there was noted tremor, peculiar gait and weakness similar to that of paresis.

The investigations from Bunge's laboratory, which are mentioned in his book on physiological and pathological chemistry (9), are worthy of attention. Lunin (10) reported experiments with mice, fed on casein, fat and cane sugar. Out of five animals used, one

lived 11 days; the others, 13, 14, 15 and 21 days, respectively, while starving animals lived only from 3 to 4 days. The addition of soda, which served the purpose of neutralizing the sulphuric acid arising from the protein cleavage prolonged the life of the animals to a certain extent; the ash of milk likewise had the same effect. Lunin explained the results as being due to a lack of organic phosphorus compounds (lecithin), and to a disturbance in the balance between the inorganic and organic food components. Subsequently, Lunin made the unusually important observation that mice could thrive very well on milk powder even after two and a half months, and therefore concluded that milk contained besides the known elements, other unknown substances essential to life. That this conclusion had already been arrived at in 1881 must appear to us as truly remarkable.[1] Socin (11), working in the same laboratory, also came to a similar conclusion in 1891. His problem required him to find out whether inorganic or organic iron is the better utilized in the animal body. Mice were kept on a diet consisting of blood serum, fat, sugar, starch, cellulose and ash (obtained from milk). The missing iron was added in the form of hemoglobin, hematin, or iron chloride, without the least effect being apparent on the duration of life. All of the animals died after 32 days, while the controls, fed on yolk of egg, starch and cellulose, were still alive after 99 days. Bunge (12) himself obtained the same results. Häusermann (13), working with rats, guinea pigs, rabbits, cats and dogs, kept on white bread and rice, could see an improvement after the addition of iron, although a normal state of nutrition could not be brought about. The experiments of Socin were confirmed by Hall (14) and Coppola (15).

Considering simple feeding experiments, we find the work of Pasqualis (16) on chickens, receiving a diet composed of 14 per cent protein, 65 per cent corn starch, 4 per cent dextrin, 4 per cent sugar, 9 per cent olive oil, 2 per cent wood chips, 1 per cent common salt

[1] Lunin concluded that "mice can live well under these conditions when receiving suitable foods (milk) but as the above experiments demonstrate that they were unable to live on proteins, fats, carbohydrates, salts and water, it follows that other substances indispensable for nutrition must be present in milk besides casein, fat, lactose and salts." This conclusion was quoted in Bunge's Physiological and Pathological Chemistry, but did not attract the attention it merited.

and 1 per cent salt mixture (in the form of corn ash). To his astonishment be observed that the animals lost weight and died—in spite of the large food intake. The first exact feeding experiments on rats were carried out by Henriques and Hansen (17). The experiments were, in the main, of short duration, lasting only from three to four weeks. During this time, the rats gained weight and showed a retention of nitrogen, but the experimental period was apparently too short to discover any food deficiency. Different findings, however, were reported by Falta and Noeggerath (18) who repeated these experiments. They fed various proteins of animal origin together with fat, sugar, starch, salts, lecithin and cholesterol. It was possible to determine the value of the various proteins, although not one of the combinations was permanently sufficient, as was apparent by the poor nourishment to be noted after varying intervals of time. The deficiencies of the diet made their appearance only after prolonged feeding. If the experiment had been terminated at the end of four weeks, the false conclusion could have been made that the diet had been adequate. The food intake was diminished, and the interesting observation was made that the eyes of the rats exhibited pathological changes, of which we shall have more to say later. Working in the laboratory of O. Frank, Jacob (19) reported some feeding experiments with pigeons and rats on a diet which at that time was thought to be sufficient. The pigeons died after a maximum of four weeks with symptoms of severe digestive disturbance, although they lived somewhat longer when casein was replaced by meat powder. Rats, on the contrary, lived longer on casein and died after 43, 73 and 125 days. Undoubtedly, the cause of death was the unsuitable composition of the diet, though Jacob considered it to be due to the uniformity and lack of stimulating substances in the diet. The same trend of thought is often met with in the older as well as the newer literature. For instance, McCollum (20) explained the dietary deficiency of synthetic diets as being due to lack of flavoring and stimulating substances, although at present he is the most eager advocate of the new ideas. Through the addition of these flavoring and stimulating substances, he was unable to demonstrate an improvement in the nutritive condition. A very interesting investigation was that made by Watson (21). He fed rats, both young and full grown, on various meat products. The young animals either died very quickly or

showed inhibition of growth, while full grown animals suffered from paresis and sterility. This last symptom was not to be attributed to lack of tryptophane. The animals whose growth had been stunted resumed normal growth on a mixed diet.

About 1906, there appeared the classical work of Hopkins and his pupils, who displayed marked clearness in their course of reasoning. Hopkins fed mice on a mixture which contained zein (one of the corn proteins) which lacks tryptophane. The young animals were able to live only 16 days, whereas upon the addition of this amino acid, life was prolonged for 14 days more. Tyrosine exerted no influence. It appeared from these results that the above diet was lacking in something else besides tryptophane. The appearance of animals so fed, as described by Wilcock and Hopkins (22) was not very bright. The animals were torpid, had cold extremities, half-closed eyes and slimy fur. At that time, Hopkins thought that tryptophane could be conceived of as the precursor of adrenaline. The chemical nature of the missing components (even after the addition of tryptophane) was not recognized at that time, although the experiments led Hopkins (23) to the prophetic statement which is reproduced here verbatim:

> But further, no animal can live upon a mixture of pure protein, fat and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissue or other animals and these contain countless substances other than the proteins, carbohydrates and fats. Physiological evolution, I believe, has made some of these well nigh as essential as are the basal constituents of diet; lecithin for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something familiar, and a substance that happens to have been tried. The field is almost unexplored, only it is certain that there are many minor factors in all diets of which the body takes account. In diseases, such as rickets, and particularly scurvy, we have had for long years knowledge of the dietetic factor, but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal quantitative factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention, but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors.

All that Hopkins says in this short paragraph applies to this very day, although from the point of view of that time it was held, for example, that lecithin was essential for life. These assertions of

Hopkins were unknown, because he offered no experimental evidence in their support till 1912.[2]

Till a short time ago, there was no lack of opinions which denied the existence of substances essential to life. We have only to recall the work of Abderhalden and Lampé in 1913 (25), although lately Abderhalden has changed his view upon the matter. He (26) conducted many feeding experiments with various proteins, hydrolyzed meat, etc., without once recognizing that these diets were lacking in something that was essential.[3]

It is true that the experiments did not last very long, and that the various constituents were not carefully purified. Incidentally, it must be noted that in these experiments short periods, in which a more natural diet was given, were frequently interspersed in order to abate for a time the distressing deficiency symptoms. Above all things, we must recognize that in dogs, the symptoms manifested because of a deficient diet, are not so pronounced as in pigeons and rats. Some time after the vitamine theory had already become familiar, Abderhalden (29) found indeed that digested casein had less value for growing animals than digested meat. He immediately assumed that glycine was the cause of the difference, whereupon he started various experiments, adding this amino acid, but naturally without any success. Similar criticism may be made of the work of

[2] For instance, Röhmann (24) is mistaken when he asserts that the ideas expressed by us originated with Hopkins. Inasmuch as we entered the field in 1911, the statements of Hopkins were unknown to us.

[3] While I was working in the city hospital at Wiesbaden, I carried out under Abderhalden's direction, some metabolism experiments with dogs. The animals were fed with edestin, gliadin, milk powder and meat, together with other usual dietary constituents. Since the experiments were considered unsuccessful, only a small part of the work was published by Abderhalden and myself (27). At that time, most of my dogs died, so that the feeding experiments had to be started anew a number of times. Even then, I made the observation that animals fed on edestin or gliadin quickly recovered on addition of milk powder or meat. I wrote to Abderhalden a number of times, that the edestin and gliadin combination was not sufficient to make the animals thrive. The symptoms which I observed then recall those noted by Chittenden and Underhill (28) in dogs fed on peas. To my letters, I received the naïve reply from Abderhalden, that in such metabolism experiments, success depends upon the manner in which the animals are taken care of. I conscientiously attempted to follow this advice, but the dogs, on such a poor diet, refused to manifest any friendship for me.

Grafe (30) who studied the protein-sparing action of ammonium salts. The same thing applies to the work of many others on animal nutrition.

One of the last opponents of the vitamine theory was the late Röhmann (31 and l.c. 24). He expressed the idea that the existence of the vitamines was seized upon only because certain investigators had had poor results with their experimental animals. The subject was treated by Röhmann in a rather illogical manner, since in the end he admitted the existence of the vitamines, under a different name. His statements, in particular, have been discussed by so many authors that we shall not consider them further here.

In spite of the great powers of observation possessed by such investigators as Bunge and Hopkins, the conception of the vitamine theory could never have attained its present importance, if a powerful impulse had not been received from clinical sources. The findings mentioned above have not been utilized in human pathology, since most clinicians are not very much disposed to apply the results of animal experiments to man. However, great interest in vitamines was justifiably aroused when the applicability of the results to human pathology and physiology was demonstrated. For a long time, long before the publication of the laboratory findings, the literature contained views upon the etiology of scurvy, rickets and pellagra which appeared to be very nearly correct. The conditions surrounding these diseases were, however, too complicated to be suitable for a direct experimental research. On the other hand, as regards beriberi, the circumstances were entirely different. Here we had to deal with a problem the etiology of which was comparatively simple, for the disease could be brought logically into causal relationship with the continued consumption of rice. Nevertheless, many years of effort were necessary for these conceptions to gain a foothold in the literature. When the first edition of this book was being written in 1913, it was still necessary for us to wage a hard fight in support of our contention. Such is not the case at present, for the characterization of beriberi as an avitaminosis has met with general recognition. At the end of this chapter, we shall discuss the history of beriberi research, since it may logically serve as an introduction to the study of the vitamines.

We shall begin here with a discussion of scurvy, although this disease really did not contribute any direct stimulus to vitamine

research. Scurvy, however, is the first disease the etiology of which was associated with a definite mode of nutrition. The reasons why scurvy gave no immediate impulse to research were to be looked for in the variety of the feeding, which might have been responsible for the onset of the disease. It was difficult to conceive of the disease as being due to a lack of one and the same substance in the diet.

It was Kramer (32), an Austrian army physician, who recognized for the first time, the existence of scurvy. In 1720 with a field army in Hungary, he was confronted with a severe epidemic of scurvy. He wrote to the authorities and to his colleagues to secure help. A shipment of dried antiscorbutic herbs was hurried to him in spite of which thousands died of this disease. He then made the following entry in his book:

> Scurvy is a terrible disease for which there is no known cure. Medication does not help, neither does surgery. Be careful of bleeding; shun mercury as a poison. The gums may be massaged, the stiff joints may be rubbed with fat—but all in vain. If one could only have available a supply of green vegetables, or a sufficient amount of the vital antiscorbutic juices; or if one could have at hand oranges, limes or lemons, or their preserved pulp or juice so that a lemonade could be made out of them; or administered as such in three or four ounce doses—then one could be in a position to cure this dreadful disease, without other help.

As we see, Kramer selected a method of treatment which could not be better chosen even today.

Bachstrom (33) recognized in 1734 that the incidence of scurvy was not due to cold weather, sea air or salted meat, but to a lack of fresh vegetables. The latter, he perceived, was the primary cause of the disease. Lind (34), in his work on scurvy, has noted many cases which were cured by administration of oranges or lemons. Cider was next to oranges in its efficacy. At that time, he made the important observation that very severe cases could be cured in 6 days, and recognized also that hard work accentuated the symptoms of scurvy. Coming now to the modern history of scurvy, we must give prominence, above all others, to the name of Barlow (35) who attributed the onset of infantile scurvy (also called, Möller-Barlow's disease) to milk which had been heated for a long time. It was only lately, in 1907, that scurvy in guinea pigs was discovered—a discovery of the greatest significance to vitamine research. The work of Holst and Frölich (36) on this subject was repeated on all sides, and their results were completely corroborated.

Research on rickets was undertaken experimentally only in late years, although Miller (37) had already associated rickets with a particular dietary even before the vitamines were known. He thought that many of his cases were due to a lack of butter, and

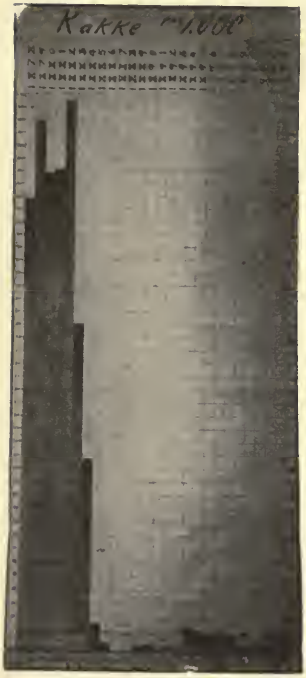

FIG. 1 FIG. 2 FIG. 3

FIG. 1. DECREASE IN THE NUMBER OF CASES OF BERIBERI IN THE JAPANESE NAVY AFTER THE INTRODUCTION OF MEAT INTO THE DIETARY (TAKAKI)

FIG. 2. THE CALORIC VALUE OF THE FOOD DIMINISHED (TAKAKI) (CF. FIG. 1)

FIG. 3. THE BODY WEIGHT INCREASED (TAKAKI) (CF. FIGS. 1–2)

consequently prescribed milk, cream, butter, egg-yolk and cod liver oil. Schabad (38) saw a marked difference between olive oil, sesame oil, and cod liver oil in their influence on rickets, and in a later work (39) he discusses the question as to the nature of the curative substance.

He was convinced that it was not a ferment and that its efficacy was not diminished after heating for one hour at 100°C. Since the war, great strides have been made in our knowledge of scurvy and rickets, and we shall discuss them in later chapters.

Beriberi is an avitaminosis about which we are best informed. Although known for hundreds and perhaps thousands of years, it is only in the last 25 years that actual progress has been made relative to its occurrence and prevention. This is perhaps due to the increase in the number of cases of beriberi, coincident with the introduction of modern machinery for rice milling. It was quite properly presumed by Wernich (40) and van Leent (41) that there existed a causative relationship between rice consumption and beriberi. In 1882, Takaki (42) proposed a change in the rice diet of the Japanese navy, so that meat, bread, fruit and vegetables were added. Since that time beriberi has almost completely disappeared.

Takaki's tables are exceptionally instructive. It is apparent from Fig. 1, that upon the elimination of the rice diet in 1882, there was an immediate drop in the number of cases of beriberi, which number subsequently remained trifling. In Figs. 2 and 3 it may be seen further, that although the caloric value of the new diet was smaller in comparison to that of the previous one (causing beriberi), there was a definite increase in the average weight of the men.[4]

Even greater strides were made in Java by Dutch investigators. Based upon very rich statistical material collected at Eijkman's suggestion by Vordermann (43) in a large number of Javanese prisons, it was possible to demonstrate that the disease was associated with the ingestion of white (polished) rice. The fluctuation in the number of cases of beriberi due to various kinds of rice is well illustrated in Vordermann's Table:

KIND	NUMBER OF PRISONERS	NUMBMR OF BERIBERI CASES	RATIO $\frac{\text{BERIBERI CASES}}{\text{NUMBER OF PRISONERS}}$
White rice	150,266	4,201	1:39
Rice with partial "silver skin"	35,082	85	1:416
Unpolished rice	96,530	9	1:10725

The findings of Vordermann were corroborated by Braddon (44) on a large number of cases in the Malay peninsula. He was able to

[4] Figures 1, 2, and 3.

show that some natives, like the races of Tamils, who live on "cured" or "parboiled" rice, remain free from this disease. This rice was so prepared that it was steamed before using; the husk came off easily, thus obviating the necessity for polishing. To convince himself of the accuracy of Braddon's observations, Fletcher (45) in 1905 undertook, in the Kuala Lumpur insane asylum, some research on lunatics, which lasted for one year. His purpose was to determine the difference between "cured" and ordinary decorticated rice. Throughout the entire perod, the diet varied only qualitatively; out of 120 patients on polished rice, 36 contracted beriberi from which 18 died, while from 123 patients on "parboiled" rice, only two developed beriberi, and these two cases were admitted as such into the hospital. In 1909, Ellis (46), working at the insane asylum in Singapore, reported similar investigations, which extended back as far as 1901. In his fruitful statistics, he showed definitely that the number of beriberi cases decreased steadily from year to year, upon gradually substituting steamed rice for polished rice. Analagous results were also obtained on healthy laborers by Fraser (47).

In 1897, Eijkman (48) (cf. Vordermann), found that beriberi is brought about by a long continued consumption of white rice, since polishing removes a substance which is protective against the outbreak of beriberi. The Dutch investigators were of the opinion that the most important part of the rice grain, the so-called "silvervlissen" (silver skin), could neutralize the toxins of white rice. Thereupon, it fell to Fraser and Stanton (49) to make clear the point that upon polishing rice, more than just the "silver skin" is lost.

Eijkman's (50) discovery of experimental beriberi marked a great step forward. This finding was made accidentally since Eijkman observed that chickens which fed upon the remains of the food used in a hospital for beriberi died of a disease which he recognized opportunely to be similar to human beriberi This discovery, which was made in 1896 (the disease was called "polyneuritis gallinarum"), made it possible to get away from experiments upon man, and assured the collection of more valuable experimental data in a shorter time.

After the discovery of experimental beriberi, Eijkman went a step further. He found that the addition of the pericarp of the rice kernel, or even the rice bran, to white rice made it possible to prevent the occurrence of beriberi in animals. For these observations, however, he did not find the correct explanation; he believed that the

starch of the grain gave rise to toxins which exerted a deleterious action on the nervous system, and that this was prevented by the addition of the pericarp. This conception should not surprise us since in those days the nature of the disease was quite puzzling. Eijkman (51) likewise made the important observation that the watery extract of rice bran possessed therapeutic properties. Phytin was found in the rice bran, but it was shown to be without effect on beriberi. He noted also that the curative substance is dialysable and not precipitated by the addition of alcohol. The observations made in 1897 were, after all, those upon which the modern research on beriberi is based. All credit is due Eijkman for having laid the foundation for the conduct of future experiments.

Grijns (52), continuing the work, was able to confirm the experiments of Eijkman completely. He was the first worker to express clearly a conception of beriberi, which holds good to this day. Grijns said that the disease developed when the diet was lacking certain substances which were of importance in the metabolism of the peripheral nervous system.[5] Curative substances similar to those occurring in rice bran were found by Grijns in a kind of bean called "Katjang-idjoe" (Phaseolus radiatus), and in meat; he also demonstrated that these foodstuffs lost their curative properties when they were heated to 120°C. These experiments were of greatest significance in the further development of the question, and they were also confirmed by Eijkman (l.c. 51). Bréaudat (54) used rice bran in the treatment of human beriberi with good results. Fraser and Stanton (55) sought to determine more definitely the nature of the substance. They found that it was soluble in strong alcohol and that it retained its activity after the removal of alcohol-soluble proteins. They made analyses of various kinds of rice and believed that a rice poor in phosphorus would cause beriberi. Thereupon, they suggested the phosphorus content as a practical indicator of the nutritive value of rice. For instance, rice which contained a minimum of 0.46 per cent P_2O_5 was to be considered harmless. However, we should not forget that Schüffner and Kuenen (56) have shown that the method of preparation of the rice diet is likewise of importance. That is, the rice should be partaken of together with the broth, particularly in the case of whole rice.

[5] Eijkman carried out a lengthy polemic on this point, but he admits now (53) that Grijns was correct.

If the broth is regularly discarded then beriberi can easily occur, even with whole rice. Based upon the findings of Fraser and Stanton, Schaumann (57) thought of beriberi as a disturbance in metabolism due to a lack of organic phosphorus compounds. This theory was likewise applied to other avitaminoses, such as scurvy and ship beriberi. It found disciples (Simpson and Edie (58)), and dominated this field of pathology till the advent of the vitamine theory. At that time, it was quite plausible to regard the great difference in phosphorus content between white rice and rice bran, and the undeserved acclaim of organic phosphorus compounds, as therapeutic factors in medicine.

In the years that followed, there appeared in quick succession a great number of experiments dealing with the chemical nature of the curative substance. Various foodstuffs were used for this purpose. Hulshoff Pol (59) showed that a watery extract of "Katjang-idjoe" beans, clarified with lead acetate, contained the curative substance. From this clear filtrate, Pol obtained a crystalline substance which he called "X-acid;" there is a lack of further information as to whether this substance possessed any characteristic physiological properties.

Schaumann (l.c. 2), who extended the list of curative substances to include yeast (which was used by Thompson and Simpson (60) in the treatment of human beriberi), investigated the influence of the already known yeast constituents. Among these he investigated the influence of yeast nucleic acid and yeast lecithin, but without obtaining definite results. Eijkman (61) showed that the active substance of yeast could be extracted with 88 per cent alcohol. Funk (62) had already noticed this before, but by this method only an incomplete extraction could be effected. Teruuchi (63) extracted rice bran with dilute hydrochloric acid, neutralized the solution, thereby precipitating phytin, then evaporated the filtrate and extracted the residue with alcohol. This extract was active and contained only a small proportion of its original phosphorus content. Similar results were also obtained by Chamberlain and Vedder (64). They found that the curative substance was adsorbed with animal charcoal and tried to develop a procedure based upon this finding, but failed. Shiga and Kusama (65) found that the active principle of rice bran was destroyed by heating to 130°C. with 0.5 per cent hydrochloric acid or with 1 per cent soda solution, but not at 100°C.

Research on the preparation of the active substance from rice polishings were also conducted by Tsuzuki (66), but with little success. Owing to the enormous content of phytin in rice bran, Aron and Hocson (67) believed that it was curative; the good results they obtained may be explained by the probability that the phytin was contaminated by some of the active substance. Research on phytin had already been carried out by Eijkman without results, and also by Cooper and Funk (68).

To summarize our knowledge of the chemical nature of the active principle prior to the introduction of the vitamine theory (till 1911), the following may be set down with certainty:

1. The substance is soluble in water, alcohol and acidified alcohol.
2. The substance is dialysable.
3. The substance is destroyed at 130°C.

When we took up the question in 1911, it was not known whether the active substance was organic or inorganic in nature, whether or not it was a constituent of proteins, nucleins or phosphatides. It was not certain that we were not dealing with a ferment, nor was it known if the substance belonged to some chemical group already described, or to some new unknown class of substances. We shall be in a position to answer a good many of these questions during the course of our discussions.

INTRODUCTORY

In the historical part, we have seen that the animal organism cannot live very long upon an artificially prepared diet. Since systematic investigations into the vitamine requirements of all classes of plants and animals have not yet been made, it is not possible to say with entire certainty that the above contention is generally true. The firmly established importance of the vitamines for the existence of certain animals and plants, organisms which are far removed from each other genetically, makes it apparent that these substances are of universal importance to life.

It is evident that there are differences in the qualitative and quantitative requirements and it may later develop that various organisms need various quantities as well as various kinds of vitamines. We shall briefly enumerate the facts that have led us to this conclusion, and point out how many types of vitamines are known up to the present. We choose to speak of vitamine types in this case, and not of definite vitamines, for as long as these substances remain unidentified, and till such time as they may be compared with each other in the pure state, it is obviously impossible to talk of their identity. Until now, we have differentiated three such types. Whether with these three types we have finally reached the possible limit, it cannot be stated definitely; however, it appears unlikely that Nature, in the variety of its manifestations, essential conditions and intensity of metabolism, should limit itself to only three types. On the other hand, it is possible that after purification, that which seemed to us a single substance might well prove to be a mixture. We may be dealing with complex substances having some chemical groups in common, and others, the significance of which has not yet been demonstrated.

It would be quite premature to propose, with McCollum (69), that only two or three vitamines exist in nature. He arrived at this conclusion as a result of his numerous rat experiments, which showed that for this species two types of vitamines were sufficient, and thereupon drew conclusions that were to be applied to the entire plant and animal kingdom. Although we are very well informed as to the food requirements of rats, compared to those of other organ-

isms, we cannot maintain that we know all that there is to be known of the requisite food constituents, as long as we shall not have obtained them (including vitamines) in the chemically pure form. This applies naturally even more to other animals, about whose metabolism we are still less informed. It is therefore of greatest importance, at this stage of our knowledge, to disregard generalizations of this sort since they are detrimental to the development of the questions that interest us.

We shall now consider the work leading to the present conception of the three different vitamine types. When we pointed out in 1912 (l.c. 62)[1] that the animal organism needs vitamine for complete nutrition, the term, "vitamine," was used only in a very general way. At that time, we drew a distinction between the antiberiberi, antiscorbutic and antirachitic vitamines, although this classification was based only upon the physical characteristics, origin and influence on metabolism. New facts have been brought to light, but this classification has remained unchanged. We knew in 1912 that the pericarp of grains contained something that was of importance in the metabolism of man and certain species of birds. Then Schaumann (l.c. 2) found that yeast and some animal extracts contained a subtance of similar nature. Furthermore, it was known that man as well as the guinea pig was in need of a vitamine as a protection against scurvy—a vitamine that was recognized by us to be quite different from the antiberiberi vitamine. In spite of the above mentioned facts and the work of Stepp (l.c. 6, 7) already referred to, and in spite of the results of vitamine research in 1911, the new ideas were not immediately applied to the general science of nutrition. The

[1] I regarded it of paramount importance, that the then ruling conception of the necessity of the lipoids or the nuclein substances was substituted by the fundamentally different vitamine theory. At the same time, I must admit that when I chose the name, "vitamine," I was well aware that these substances might later prove not to be of an amine nature. However, it was necessary for me to choose a name that would sound well and serve as a catchword, since I had already at that time no doubt about the importance and the future popularity of the new field. As we have noted in the historical part, there was no lack of those who suspected the importance of still other dietary constituents, besides those already known, for the nutrition of animals. These views were unfortunately unknown to me in 1912, since no experimental evidence had appeared in their support. I was, however, the first one to recognize that we had to deal with a new class of chemical substances, a view which I do not need to alter now after eight years.

only investigation on this subject was the classical work of Hopkins (70) which appeared in 1912, demonstrating that the addition of a small quantity of milk to an artificial diet induced growth in rats. The amount of milk was so small as to be negligible, as far as its energy factor was concerned. In these experiments, there was no attempt to differentiate between the various vitamines, since milk contains all that is necessary to life. While the vitamine conception had at that time attained a definite standing in England (not without vigorous effort), the dissemination of these ideas in other countries met with but poor success. For instance, Abderhalden and Lampé denied the existence of vitamines in 1913, and Röhmann even in 1916. In the United States there appeared simultaneously with the beginning of vitamine research the very important work of Osborne and Mendel (71) on the artificial feeding of rats, which greatly advanced our knowledge of the food value of the various kinds of proteins. These investigations were indeed the first to be carried out with such carefully purified proteins and over such a long period of time (more than one year, one-third of the lifetime of a rat). These experiments served two purposes, first, to determine the nutritional value of various proteins, and secondly, to determine how long rats can live on an artificial diet. At that time, we emphasized the fact that for such investigations it is particularly important to provide the animals with vitamines, if clear results are to be obtained. Besides this, we showed that in many nutrition experiments the diet was in some unknown manner contaminated by vitamines which were responsible for the length of time the animals survived. This was later shown to be true; for example, we could demonstrate the presence of some nitrogen-containing impurity in milk sugar; the same is true of other products derived from milk. In the experiments of Osborne and Mendel, the diets, taken as a whole, were obviously lacking in vitamines, since many of the animals died suddenly, or they would have died if the diet had not been changed quickly. Young animals lived for a certain time, but mostly failed to grow.

In another investigation, Osborne and Mendel (72) described experiments in which rats were fed on a mixture that could have been thought of as fat-free. It contained, among other things, protein-free powdered milk extracted with ether. In this case also, normal growth was obtained. The same authors then analyzed the

protein-free milk for its inorganic constituents, and prepared an artificial protein-free milk having the same composition; this also permitted of good growth. In a later communication, these investigators (73) pointed out that the results were not so favorable with the artificial preparation. Their animals lived from 114 to 277 days, but after this they died without the post-mortem giving any plausible reason for death. In one case particularly, the addition of natural food was ineffective in preventing death.

Hopkins and Neville (74) reported that they attempted to replace milk by a preparation made according to Osborne and Mendel. They stated that when they used purified lactose, which had been prepared from milk, the animals failed to grow. McCollum and Davis (75) found that rats, weighing 40 to 50 grams, could grow normally for about three months on the Osborne-Mendel diet. At the end of this time the animals stopped growing, but were in good general health. In the light of our work on vitamines, the authors came to the conclusion that the failure to grow was not due to lack of salt, fat and phosphatides, but to lack of vitamines. They found the necessary substances in egg-yolk and butter, especially in the ether-soluble portion. Besides this, they found that rats on the above diet produced very little milk, and that the young were stunted. Normal growth was restored upon the addition of the ether extract of eggs or butter.

Almost simultaneously with the work of McCollum and Davis, there appeared the paper of Osborne and Mendel (76) in which they confirmed their earlier findings. As a result of these studies, they showed that artificial milk, like the natural protein-free variety, is not sufficient for growth. To obtain adequate growth, either milk powder or butter had to be added.

In later publications, McCollum, as well as Osborne and Mendel, disregarded the importance of the antiberiberi vitamine for growth more and more, and reiterated the existence of a specific growth substance in certain fats, like butter, egg-yolk and others. Little by little, the entire vitamine structure became shaky, especially after Osborne and Mendel (77) had started their studies on butter. By centrifugation, they separated butter into three fractions. They reported that the pure butter fat, supposedly nitrogen and phosphorus free, still retained its activity. In particular, they were able to cure ophthalmia (keratomalacia, a disease of which we shall speak

later) in rats kept on an artificial diet. These experiments were intended to show that the growth substance could not be a vitamine. We do not yet know the chemical nature of the substance in butter, but there is nothing to disprove the conception that this substance contains nitrogen and may be classified as a vitamine, especially when it is remembered in what small amounts it may be active.[2]

Working with Macallum (81) on the fractionation of butter, we demonstrated that when we followed the procedure of Osborne and Mendel, and dissolved large amounts of butter fat (about 12 kilos) in acetone and shook out this solution with dilute hydrochloric acid, we found 23.4 mgm. of nitrogen in the extract; after hydrolysis of the fatty residue with dilute hydrochloric acid, an additional 22 mgm. was obtained. It is quite obvious that it is impossible to remove all the nitrogen from butter with the above procedure. In spite of these findings, it is true that butter is poor in nitrogen and therefore it must contain a substance that is active in minimal amounts. McCollum and Davis (82) endorsed this opinion, inasmuch as they doubted the absence of nitrogen in butter. In order to answer the question, Osborne and Wakeman (83) conducted another experiment with butter and found that it contained traces of nitrogen and

[2] Later on, McCollum and Kennedy (78) sought to introduce the classification, "water-soluble B" for the antiberiberi vitamine and "fat-soluble A" for the antirachitic vitamine; lately, Drummond (79) used the term "water-soluble C" for the antiscorbutic vitamine. Regarding this matter, I have attempted to show (80) that these designations are incorrect, chemically and logically. It would be totally inaccurate to differentiate substances extracted from complicated mixtures by their solubility in certain solvents. It would seem to be of importance to replace, in the English publications, such designations as "growth-promoting, water-soluble, accessory B factor," by something more simple. For this purpose, I suggest, at least for the present, the adoption of the following nomenclature:

Vitamine B for the antiberiberi vitamine
Vitamine A for the antirachitic vitamine
Vitamine C for the antiscorbutic vitamine

These letters are already used very often in the English literature, and the nomenclature to be used in this book is suggested as being simple and time saving, particularly in indexing the literature. Since this has been written, a note by Drummond (80a) has appeared in which the same kind of nomenclature is proposed, except that he suggests "vitamin" instead of "vitamine" for the English scientific literature. I cannot agree to this change since I still believe in the nitrogenous nature of these substances.

phosphorus. The question as to the nitrogen content of butter has not been touched upon for some years, since the solution of the problem could only be obtained through a purification of vitamine A. In this connection, the amount of lecithin in butter, based upon the phosphorus content, was calculated to be 0.017 per cent by Wrampelmeyer (84) and from 0.04 to 0.07 per cent by Supplee (85); the latter also demonstrated the presence of choline, trimethylamine, and ammonia.

While Osborne and Mendel, as well as McCollum, regarded butter and certain other fats as the only growth-promoting substances, Funk and Macallum (86) went to the opposite extreme, stating that vitamine B was the only growth-promoting substance. It appears from the newer investigations, as we have always believed, that vitamine B is far more important to life and is required in greater amounts then vitamine A. Nevertheless, both substances are necessary for growth as has been developed by the above controversy. The Funk-Macallum experiments showed that butter, when added to an artificial diet, does not cause growth, and it appeared that an addition of yeast was imperative. To be sure, there was then no apparent difference in the efficacy of butter and lard, but it should be said here that in our investigations we found it necessary to add a large amount of yeast in order to obtain good results; fresh yeast worked better than dried yeast. It is not impossible that yeast, in the fresh condition, contains some vitamine A; on the other hand, in the light of the results obtained by Daniels and Loughlin (87), it is possible that some fats, till now looked upon as vitamine-free, contain enough of this vitamine to stimulate growth in rats.

In this manner, the existence of two vitamines, A and B, was definitely established. In addition, it became apparent that the substance playing the greatest part in the growth of rats is either identical with the antiberiberi vitamine or belongs to the same type. Coming now to vitamine C, its individuality and its differentiation from the other two vitamines was determined in the following manner. While we find vitamine C often associated with vitamine B in Nature, there are products such as egg-yolk and cereals, which are very poor in vitamine C but especially rich in vitamine B. The evidence of their dissimilarity may be seen more clearly in the work of Seidell (88), who showed that vitamine B could be adsorbed quantitatively with Lloyd's reagent (fuller's earth). Harden and Zilva

(89) then showed that from a mixture of autolyzed yeast and orange juice, substances frequently used as a source of both vitamines, vitamine B could be completely removed by adsorption with fuller's earth, while the presence of vitamine C could be demonstrated in the filtrate. These findings have been corroborated by Byfield, Daniels and Loughlin (90) incidental to some other work. Vitamine C is the most unstable of the vitamines, although it appears to be more stable in juices that are slightly acid. In association with these facts, there is the discovery of Fürst (91), who was the first one to show that if grains are allowed to germinate a formation of vitamine C takes place, which disappears again upon drying and reappears upon the addition of water. We have thought (l. c. 62) that in this observation there was a possibility of finding a genetic relationship between vitamine C and vitamine B. Through progress in the methods of demonstrating the presence of vitamine B, it may be possible to investigate the question, if with the formation of vitamine C there is a corresponding diminution in vitamine B. The relationship between these vitamines has become more interesting, since Osborne and Mendel (92) showed that known antiscorbutics, such as fruits, always contain a definite amount of vitamine B. Although we know that this vitamine has no effect upon scurvy, the reverse of this, the influence of vitamine C on beriberi, is not sufficiently established. It might be possible for instance, that for the cure of scurvy very small amounts of vitamine C are necessary, whereas to influence beriberi similarly much larger doses are required. This matter must be cleared up by further research.

We must also put forth the question whether, with the enumeration of the above three vitamines, we have exhausted all the possibilities. There is no definitive answer to this at present, but it is frequently touched upon in the latest investigations, and it came into prominence through a review of the available data by Mitchell (93), pointing to the idea that vitamine B is different from the growth-promoting substance. In spite of this, we must note that the conclusions of Mitchell are not indisputable. He compared earlier work done with comparatively inefficient methods, with newer work that is more liable to be accurate, and consequently it is not to be wondered at that he found a difference; besides, he compared extracts made from different materials. Somewhat later, there appeared the first experiment on this matter of Emmett and Luros (94), which at least had

the advantage that the source of material (unpolished rice) was the same in the comparison of both characteristics. We shall comment upon the questions referred to, particularly the vitamines, in greater detail, but at this point we wish to emphasize that the functions of growth and the curative action on beriberi were tested on two different animals, the pigeon and the rat. Heated unpolished rice still retained its growth promoting property, although to a somewhat lesser degree, while its influence on pigeon beriberi was completely destroyed. We can find many explanations for this behavior without necessarily accepting the existence of two vitamines of the same type. Above all, we do not know just what are the vitamine requirements of these types of animals. We shall see furthermore that all the known facts point to the idea that, although it shall have been demonstrated that both substances differ from each other, they would still be related chemically. In this connection, it might be shown that various types of animals can utilize the different stages of vitamine cleavage with varying degrees of success. This could proceed, on the one hand, through a difference in synthetic abilities and, on the other, through certain specific symbiotic intestinal flora.

A similar question is brought up in reference to vitamine A (antirachitic vitamine). Mellanby (95) holds the view, that in the etiology of rickets, that vitamine which according to our nomenclature must be called antirachitic vitamine (vitamine A) is of importance. It is found in fats such as butter, egg-yolk, and cod liver oil. Whereas Mellanby worked with young dogs, Hess (96), working with rachitic children, found that milk fat had no effect, while cod liver oil was found to be beneficial. From this, Hess drew the conclusion that the growth-promoting vitamine found in butter was different from the antirachitic vitamine. Here also, both substances belong to the same type, and we must bear in mind the main question whether the vitamines, like other natural substances, do not also deviate from each other somewhat chemically, in spite of the possibility that they play the same or very nearly the same rôle in physiology.

Much more important and interesting are the experiments which deal with the part played by milk and milk products. We are sometimes almost tempted to assume that milk, aside from the vitamines already mentioned, contains still other essential substances. This applies not only to milk, but also to products made from milk,

such as casein, lactalbumin, and partly also to lactose. Obviously another conclusion is possible here, namely, that milk contains the most suitable combination of amino acids for the animal body. It was pointed out by McCollum and Davis (97) that heating of casein for one hour, at one atmosphere in an autoclave, materially decreases its food value; Funk and Macallum (98), attempting to confirm this observation, found that the facts were somewhat in accord, but that the diminished nutritive value of casein could be remedied by the administration of fresh orange juice.[3]

We believed, at that time, that heating of the casein destroyed the adsorbed C-vitamine, and our view was strengthened by the later work of Harden and Zilva (100) and Drummond (l.c. 79). We (101) had already been able to show that beriberi in pigeons could be cured by the addition of the vitamine fraction of lime juice, although we were not certain if this effect was not due to the presence of vitamine C. Still later, Osborne and Mendel (l.c. 92) showed that the favorable influence of orange juice on the growth of rats was due to the presence of vitamine B. This observation was further confirmed by the work of Byfield, Daniels and Loughlin (l.c. 90), in which they showed that orange juice treated with fuller's earth exerted no growth-promoting influence either on young or grown rats, in spite of still containing vitamine C. Till now, there has been no explanation of why, when the diet already contains apparently sufficient vitamine B, upon the addition of more of this vitamine the organism should respond with an acceleration of growth. There is a possibility here that orange juice may contain a new vitamine which, together with B vitamine, is adsorbed by fuller's earth.

Osborne, Wakeman and Ferry (102) showed that certain types of proteins, for instance, edestin, manifest a greater capacity for adsorbing vitamine B than do other proteins, and that the latter, by means of thorough washings, could not be freed from it. It would be plausible to assume that casein, as well as lactalbumin, might show a selective adsorptive capacity for some new vitamine. As we shall show towards the end of this book, the acceptance of a new and important amino acid or of a new vitamine appears inviting for the elucidation of the etiology of certain pathological conditions, and also for the explanation of the difference in food value between proteins of vegetable and animal origin.

[3] Hogan (99) found that heating of proteins, casein in particular, does not impair their effectiveness.

In a great number of experiments, Osborne and Mendel (103) showed that the nutritive value of lactalbumin is much greater than that of casein, and they attributed it to the nature of the particularly favorable amino acid composition of this protein.[4] Edelstein and Langstein (104) recently arrived at the conclusion that the superiority of mother's milk over cow's milk for the child is due to the greater content of lactalbumin in mother's milk.

In a series of papers, which are not altogether clear in their meaning, Emmett and Luros (105) sought to show that while lactalbumin of itself was a complete protein for growth, the specially favorable results obtained must be attributed to the influence of the protein-free milk added at the same time. In this, they coincide with the view of McCollum, Simmonds and Parsons (106). In other words, the favorable influence of lactalbumin on the growth of rats was to be attributed to the presence of a vitamine in the protein-free milk or the lactose. This vitamine was supposed to be different from vitamines B and A.

Emmett and McKim (107) have also assumed the existence of two vitamines in yeast—one that cures beriberi and one that promotes growth in animals. They reached these conclusions from adsorption experiments with fuller's earth. They showed that the activated fuller's earth was protective against beriberi, but did not influence the weight of the animal. Such experiments, unless well controlled, can have no real significance, considering the possibility of incomplete adsorption, the relative amounts used and other factors.

Sugiura and Benedict (108) point out that certain diets with the addition of yeast serve to increase the growth of young rats, although the mothers appear to be lacking in milk. They conclude therefore that for this purpose a special vitamine is desirable. As an illustration of this, the example is given that an addition of casein exerts a much greater influence than a simple addition of extra protein. In one particular case it was shown that purified casein could not be replaced by purified meat. In a second experiment, they diminished the amount of the added yeast to 0.5 per cent of the total diet and it therefore appeared not impossible that the failure of milk production

[4] Later, these investigators showed that this is true only when protein-free milk is used as source of vitamine B. This addition seemed to have the effect of completing the value of that particular protein.

was due to an insufficient amount of vitamine B. Unfortunately, these investigators failed to determine whether an addition of B-vitamine would not stimulate milk secretion. Invariably, all of the above mentioned workers stressed the possibility of finding a still unknown vitamine, especially in milk.

In conclusion we wish to mention that besides other possibilities of clearing up the etiology of pellagra, we have already intimated (l.c. 62) that there might be a specific antipellagra vitamine—a view held as well by Goldberger (109). This vitamine could be a substance easily adsorbed from animal protein as we pointed out on page 43. Still, for these or for other conceptions of pellagra, no real experimental evidence is at present available.

After having discussed the definitely established types of vitamines, we shall take up the vitamine requirements of various organisms.

PART I

The Vitamine Requirements of Plants and Animals

CHAPTER I

The Rôle of the Vitamines in the Vegetable Kingdom

It was quite clear from the start that the importance of the vitamines was not limited to animal life only, but was applicable to all living matter. At present, the view is held that the animal organism does not possess the ability of synthesizing these factors, and in this respect it is dependent either directly or indirectly on plant life. We must determine, however, which plants, and especially what parts of plants, possess the power of building up the vitamines out of simpler combinations. Furthermore, we should like to know what rôle these substances play in plant physiology. We can immediately assert that although the work on this aspect of the subject is still in its infancy, not all plants apparently are able to build up the vitamines (this is perhaps not true of all vitamines).

Upon more careful consideration of the higher plants, we find the vitamines localized in two divisions, in the seeds and in the green leaves, parts of the plant which at certain times are of particular importance in its metabolism. We find, with few exceptions, the greatest amount of vitamine B in seeds, together with varying amounts of vitamine A. It is quite apparent that in seeds, the analogue of milk and eggs in the animal kingdom, all the substances are present which are necessary for the sprouting of the new plant. The vitamines play a great part in this process, yet we are dealing here only with conjectures. As soon as seeds are planted under conditions of proper moisture and temperature, the ferments, which had been in a quiescent state, commence to function. These ferments begin to marshal the stored up reserve substances. In this instance, we may also consider the vitamines as reserve substances, and we may conceive that they too undergo some chemical changes through the influence of ferments. As an analogy to this example we already know that vitamine C is generated upon the germination of seeds. All other phenomena, like the division of the nuclei of cells, remain unknown to us, although we are soon aware of the appearance of the first green shoot. What part the vitamines play in this case is not definitely known. Perhaps they are of importance

in the first stages of metabolism; perhaps they are responsible for the sudden onset of cell division. In organisms which multiply by conjugation or copulation, the vitamines or similar substances exert some influence perhaps in the subsequent nucleus and protoplasm partition. When we compare the quiescent with the sprouting seed, we see the difference between the living and the dead tissue, although the gross chemical compositions in both conditions are not far different from each other. The entire difference may be due to the formation of an active vitamine modification. It is evident that no great progress is made when an unknown matter is explained by something equally unknown. It may nevertheless be of value, as far as future investigations are concerned, to point out in which class of chemical bodies the substances essential to life are to be sought.

Clark (110) described the influence and characteristics of a substance which he called "Oöcytin," and which was supposed to be able to bring about the formation of the fertilization membrane of sea urchin eggs, previously treated with strontium chloride. This substance was prepared in powdered form from centrifuged and defibrinated ox blood. The powder gave the reaction of protein, purine, and pentose, and contained only a small amount of phosphorus. It was inactivated by heating to 73° to 80°C., or by long contact with alcohol. Similar substances were demonstrated in the sperm of the sea urchin by Robertson (111). These or other active substances naturally play a similar part in the plant kingdom, although they are for the moment unavailable for physiological-chemical work.

The question as to the ability of certain types of plants and particular parts of plants to build up vitamines is still far from solution. In higher plants, for example, there are three possibilities:

1. In seeds, stored up vitamines suffice to stimulate further synthesis of vitamines in the growing parts of the plants.

2. It is possible that the small amount of vitamines in seeds suffices to carry the plants along to a certain stage, from which there is a symbiosis with certain microörganisms which provide the plants with vitamines.

3. It is possible that some of the plant structures, for example, the leaves, possess the ability to synthesize certain vitamines.

The subject of this chapter serves merely as a suggestion of the important rôle of the vitamines in plant life, without which knowledge of the vitamines can never be complete. The very few facts mentioned here should serve to stimulate further work in this direction.

YEAST

Since the discovery of its curative influence on beriberi by Schaumann (l.c. 2), yeast appears to be the best material available for the chemical study of the vitamines. Of all natural products, it seems to be the one most rich in vitamine B and therefore is worthy of special attention. One could be tempted to assume that since yeast cells are so rich in vitamines and possess such an energetic metabolism of their own, they should be able to prepare their own vitamines. This is, however, only partially the case, as has been already shown in some older neglected work which has recently been repeated, and confirmed. Pasteur (112) made the observation in 1871 that the capacity of yeast-cell growth depended very much upon the size of the inoculation. He found also that addition of certain organic substances accelerates the fermentation of certain types of yeasts. It was Wildiers (113) who correctly understood Pasteur's observation and developed it further. It may well be said that Wildiers foresaw the existence of the vitamines as far back as 1901. His important studies have demonstrated that upon the addition of a small quantity of sterile yeast extract to a nutritive solution, containing an ammonium salt as its sole source of nitrogen, a small inoculation is sufficient; the growth of the cells being measured in this case by the CO_2 evolved. He recognized that this growth stimulus could not be attributed to the presence of the hitherto known factors, and named this new substance "bios." Its characteristics were not so far different from that which we have come to know as B-vitamine. It was soluble in water and also in 80 per cent alcohol, and insoluble in ether. It was dialyzable and could not be precipitated by the well-known precipitants, phosphotungstic acid included. The substance was found to be stable in acid medium but on heating for a short time with alkali it was destroyed. The investigation of the resistant qualities of this substance gave no clear results. The presence of bios was never shown in the decomposition products of egg albumin

split by acids, and likewise not in yeast ash. The experiments by Wildiers were then confirmed by Amand (114) who demonstrated that the failure of the yeast to grow, with just a slight inoculation, was not due to the toxicity of the nutritive solution. Devloo (115) reported that he isolated pure bios from lecithin. From his own data it is evident that his preparation was not very active, and at present we can say with certainty that the activity noted was due to the contamination of the lecithin with vitamine. In this connection, it has been also stated that lecithin is curative for beriberi; we shall have occasion to refer to this matter later.

We should not think that the important conclusions of Wildiers found immediate acceptance in the literature. For example, H. H. Pringsheim (116) took the stand that the bios of Wildiers was nothing else than protein material, which was best used in the form that is found in yeast cells themselves. This statement of Pringsheim's was introduced into the literature, and therefore the conclusions of Wildiers were given no further attention. For instance, Rubner (117) said that the growth of yeast began only when there was a certain relationship between the amount of food and the number of yeast cells, an excess of food acting as a stimulus for yeast growth. From time to time, however, reports appear in the literature dealing with the observation of Wildiers. Vlahuta (118) prepared a peptone from beer yeast, with cold sulfuric acid, which could give rise to fermentation. Kurono (119) found that a vitamine extract, prepared from rice polishings, added to Hayduck or Nägeli solution, accelerated to a greater extent the growth of yeast cells and also the degree of fermentation, than when peptone or asparagine were added. Brill and Thurlow (120), on the contrary, could obtain no increase in the growth of yeast cells with rice polishings. The ability of certain brewery residues to accelerate fermentation can be attributed to the presence of vitamines. Moufang (121) made the observation that dead beer or baker's yeast exerted a marked catalytic action on fermentation. Saito (122) was of the opinion that certain chemical substances were important to allow of a complete development of the reproductive capacity of yeast. Bokorny (123) observed an increase in weight of yeast when it was grown on urine containing sugar (we shall see later that urine contains vitamine). Vansteenberge (124) studied the influence of autolyzed yeast upon the growth of yeast and lactic acid bacteria, and found that above a certain

optimum concentration, this autolysate is no longer active and even inhibits somewhat the growth of the cells—a finding that is of greatest importance for our arguments. He found later that the autolyzate must be diluted to obtain the best results. Leucine, tyrosine, and asparagine have an inhibiting influence on the growth of yeast cells but not on the lactic acid bacteria. Recently Lampitt (125) has shown that the increase in the quantity of yeast cells depends on the original number present. Evidently, this observation is to be explained in this way: that due to excessive inoculation, a number of dead cells are implanted at the same time and that the living cells can utilize the vitamine set free.

Since the growth of yeast cells might be utilized as a means for the determination of antiberiberi vitamine, systematic investigations were undertaken during the last two years for the purpose of studying the vitamine requirements of these cells. It was expected in this way to measure vitamine B, but we shall presently show that this is not the case. In spite of the possibility that the substance promoting the growth of yeast belongs to the type of B-vitamine, newer work has shown that the substance promoting the growth of yeast is not identical with vitamine B. It is possible, however, that for yeast, an organism characterized by greater synthetic ability, the cleavage products of vitamines might be sufficient, whereas in higher animals, on the contrary, the entire vitamine complex must be administered.

In 1912, the author, using the fermentation method, endeavored to show that vitamine could act as a co-ferment, but without success. Abderhalden and Schaumann (126) were apparently more fortunate and they described a phosphorus-free substance, obtained from yeast, which could favorably influence the fermentation of certain sugars. Abderhalden and Köhler (127) likewise described the growth-promoting influence of some yeast fractions on the yeast cells. The same authors also investigated the influence of yeast extracts on growth of Flagellata (Colpoda) and Algae (Ulothrix) with positive results. A whole series of experiments on this subject has lately been published in the United States. The method of R. J. Williams (128) denoted progress in that it substituted for fermentation a direct observation on the growth of yeast cells. The method is as follows: A hanging drop is prepared so that it contains the least number of yeast cells (one if possible) obtained by

inoculating Nägeli's solution with a needle-point of yeast culture, preparing at the same time the necessary controls. The cells are counted after 5 to 6 hours and again after 20 to 24 hours. From 20 to several thousands of cells could develop from a single cell in 24 hours, depending upon the vitamine concentration. Williams undertook some problems with the help of this method, for example, the protective influence of fat upon the destruction of vitamines by alkali. Vitamine extracts, prepared with acid or alkali, appeared to be best utilized by yeast. The greatest influence was obtained by a preparation made by shaking yeast with fuller's earth. The active substance was also shown to be present in the alcoholic extract of protein-free milk, wheat embryo, and pancreatin, and in the decomposed phosphotungstic acid fraction from yeast. Casein and lactose were likewise active, although in the former the activity was not attributed to the amino acids but to the small amounts of vitamines present. The active substance was partly destroyed by heating in the autoclave for a half hour at one atmosphere. An alcoholic extract of egg-yolk was active while the ether extract containing vitamine A was inactive. The C-vitamine, as we shall note later, appears to have no influence on the growth of yeast. Bachmann (129) investigated the behavior of two kinds of yeast, one apparently "Saccharomyces cerevisiae" and the other, isolated from fermented pears. These two varieties showed a different behavior towards the vitamine solutions. One of the yeasts grew on the surface and was less dependent upon the vitamine addition (in this she indicated the possible analogy of green leaves and influence of oxygen). The Bachmann method is as follows: A yeast suspension in a liquid nutritive medium is placed in a sterile fermentation tube, and the CO_2 evolved is measured after a definite incubation period, being compared with controls in which no vitamine was added. Orange juice, yeast extract, peptone, Liebig's meat extract, honey, certain vegetable extracts and milk were tried as a source of vitamine, and the results were for the most part the same as had been found in animal experiments. Certain discrepant results could be explained by the greater sensitivity of the method. Pasteurization and sterilization decrease the activity of milk. It was also interesting in this work to note the different behavior of the two kinds of yeast as to their dependence upon the vitamine addition, one requiring more than the other. Abderhalden and Schau-

mann (l.c. 126) developed a somewhat similar method simultaneously with the American investigators.

There seems to be no unanimity as to whether or not yeast requires extraneous vitamine for growth. Thus, Fulmer, Nelson and Sherwood (129a) believe that yeast does not require vitamine B for growth. These authors were able to grow yeast on a synthetic culture medium supposedly free from vitamine. However, their findings are not as definite as their conclusions. Nelson, Fulmer and Cessna (129b) have found that if the brewer's yeast in a synthetic diet was replaced by yeast grown on a synthetic medium in the absence of vitamines, the growth of rats proceeded normally, showing that the above yeast synthetized its own vitamine. Working with pigeons, Harden and Zilva (129c) also found that yeast synthetizes vitamine on a synthetic medium but to a lesser extent than in the presence of vitamine. Funk and Dubin (129d) were unable to corroborate the findings of Fulmer, Nelson and Sherwood, using the medium F described by the latter. Poor yeast growth was obtained in the absence of vitamine while a marked improvement was noted when vitamine was added. These same conclusions were reached by Eddy, Heft, Stevenson and Johnson (129e). MacDonald and McCollum (129f) maintain also that they can grow yeast on a pure nutrient medium in the absence of vitamine B. However, they obtained better growth with this vitamine added. This subject became a matter of controversy between Ide (129g) and MacDonald and McCollum (129h), the former maintaining that yeast can grow in two ways—slow growth without bios and rapid growth in the presence of bios, while the latter hold that there is no necessity for assuming the existence of a specific substance promoting the growth of yeast. We personally are in accord with the view of Ide that at least certain yeast species do require a specific substance for growth. A number of procedures such as improving the inorganic moiety of the medium and the addition of glucose or amino acids may give slight increase in growth but not to be compared with that obtained on the addition of vitamine. Until we know how many new cells can grow out of a few broken down cells, this question, as to whether yeast can grow without vitamine, must be left open. From the foregoing, it would seem that yeast cells can produce vitamines, if an original stimulus is provided.

BACTERIA

While yeast extract was used in 1904 by Bertrand (130) for the growth of *B. xylinum*, it did not occur to anyone that this nutritive medium possessed such special characteristics. Most bacteria, however, thrive well on the commonly used media. With some organisms on the contrary, difficulties arise and consequently the method of Noguchi (131), recommending the addition of a small amount of testicular substance for the cultivation of spirochetes, denotes important progress. What was responsible for the stimulating effect of this tissue? The question remained unanswered till the war came and the bacteriological laboratories were stripped of their customary sources of supply of nutritive media. It became necessary then, above all things, to replace Witte's peptone and nutrose. Investigators found this difficult, till systematic work on this subject was undertaken. On the whole, it may be said that most of the nutritive media used before the war very likely owed their favorable influence on the growth of bacteria to a small amount of vitamine present therein. This conclusion was not apparent immediately, since many investigators thought that bacteria could grow on a pure synthetic medium. For instance, Vedder (132) cultivated the meningococcus on starch prepared from cornmeal. Doryland (133) reported success with pure synthetic media, which evidently contained no vitamine. These experiments may be explained in that the inoculation was perhaps so great, that, similar to yeast, growth took place. Pieper, Humphrey and Acree (134) reported great success with synthetic media, while Lockeman (135) considered that the factors necessary for the growth of the tubercle bacillus were asparagine, as a source of nitrogen, glycerol and inorganic salts, phosphorus, potassium and magnesium. Nevertheless, for other microörganisms, other requirements appeared to be necessary, and Bainbridge (136) believed that certain microörganisms could not grow with protein as the only source of nitrogen. This view was also maintained by Sperry and Rettger (137). Robinson and Rettger (138) compared, in this connection, a protein obtained by ferment action (which he called "Opsin"), with the products of protein acid hydrolysis. Decolorization of the opsin by animal charcoal decreased the value of that nutritive medium. Of the proteins tested, the products of casein acid hydrolysis gave the best results, but not so good as opsin. Extracts of beef worked very well.

It was of greatest importance to this entire subject that the presence of vitamines in typhus bacilli was detected by Pacini and Russel (139). They proceeded from the observation that in the typhus convalescence period there is often noticeable quite an increase in growth. The bacilli were grown on the usual vitamine-free Uschinsky nutritive medium, and consequently they must have been able to synthesize the necessary vitamine. Both the remaining medium and an acid-alcoholic extract of the bacteria were administered to rats on an artificial diet, whereupon normal growth took place. Since the presence of vitamines in bacteria was demonstrated in this way, it was plausible to assume that for certain types of bacteria, a vitamine-containing medium would be of value. Such a preparation was introduced by Mackenzie Wallis (140); it consisted of casein, peanut flour and soda, and served as a substitute for nutrose in the composition of Conradi-Drygalski medium (for the typhus coli group). The bacteria grew well on the medium and the success was attributed to the presence of vitamine in the globulin fraction of the flour. A similar preparation, introduced by Huntoon (141) was made from meat and eggs. In the description of this preparation it was stipulated that all filtration, whether through filter paper or cotton, must be avoided. A yeast extract as culture medium was also described by Ayers and Rupp (142).

Meningococcus. The conditions for the growth of this micro-örganism were systematically studied by Miss Lloyd (143). She found that growth was impossible without vitamines. Although amino acids were the chief components of the nutritive medium, it could not be utilized except when a voluminous inoculation had been made. In this way it was possible to note a difference in the behavior of different laboratory stock toward vitamines—older laboratory stock needed no special vitamine addition when a sufficient amount of amino acids was available, while newly isolated bacteria could not be cultivated without vitamine. Between the necessary amounts of vitamine and amino acids, some relationship is apparent, and therefore it is held that the rôle of the vitamines in the nutrition of the meningococcus is traceable to the acceleration of the reaction velocity of the proteolytic processes. A further function is attributed to the vitamines in that the assimilation of iron, calcium, phosphorus and iodine is facilitated. The vitamine in question was soluble in water and alcohol and somewhat thermo-stable; it could stand heat-

ing for 45 minutes at 120°C. This vitamine could be easily adsorbed by filter paper but not by glass wool. It was shown to be present in blood and in milk (and to a smaller extent in serum). On this basis, a blood agar was prepared (144) which, in spite of the coagulation of its protein by heat, still contained vitamine, but could not be subjected to filtration. The precautions to be observed as regards filtration pointed to the probability that they were dealing with two vitamines, both of which were necessary and one of which was either slightly soluble in water or not at all. The latter could be adsorbed by protein, and of special interest, is the fact (145) that the meningococcus, after from one to ten re-inoculations, does not need any more vitamine additions, and this is apparently the reason why the importance of vitamines for bacteria was not recognized sooner. At the same time, somewhat similar experiments were carried out by Gordon and Hine (146), and also by Flack (147). These investigators were able to show that serum albumin, hemoglobin, fibrinogen, legumin and human blood accelerated the growth of the meningococcus on common agar, while the addition of glucose was without any effect. Among others, they recommended trypagar, prepared from pea flour and digested ox heart. The best results were obtained with an agar containing bouillon and an extract of cooked pea flour, or the fractions obtained after removal of starch and dextrin by alcohol. Extracts of raw and cooked wheat embryo showed a similar ability to promote growth and to prolong viability; these authors believed that pea flour contained a vitamine acting on growth, while that from wheat influenced the viability. Shearer (148) also investigated the influence of some extracts. He found the active substance in nasal mucous and believed that it acted not as a usual dietary constituent, but as an activator. The substance was soluble in water, less soluble in alcohol and not at all in ether; it was unaffected by heating with strong hydrochloric acid for 12 hours. Besides the meningococcus, it was active for pneumococcus, *B. typhosus*, *B. coli communis* and other pathogenic bacteria. In the continuation of his work, Shearer (149), noted that the cerebrospinal fluid contained a substance which is still more active than blood or nasal mucus. This peculiarity explained the rapid growth of the meningococcus in the brain tissues and in the membranes and cavities of the central nervous system. In this case too, the action was observed not upon the meningococcus only, but also upon other

pathogenic bacteria, like pneumococcus, *B. typhosus*, *B. coli communis*, certain intestinal streptococci and throat bacteria. The proof that the vitamine was actually necessary was not available, acceleration in growth only being demonstrated. Eberson (150) likewise found that a yeast extract could increase the viability of a meningococcus culture for more than a month. The medium consisted of agar, peptone and potassium phosphate. The yeast was first extracted for two hours at not more than 100°C., and then sterilized for a half hour at one atmosphere.

Gonococcus. Cole and Lloyd (151) carried out vitamine experiments on gonococcus. Tryptamine blood (tryptic digestion product of casein) was used for cultivation. The gonococci cultures required a suitable ion concentration, a considerable amount of free amino acids and two kinds of vitamines to permit of favorable development. One of these, easily adsorbable, is found in blood corpuscles and possesses the ability to promote growth; the other occurs in tissues, is relatively little adsorbable, and has the peculiarity of bringing about a secondary plentiful growth. The method of demonstration of both vitamines is given in great detail. They are precipitated by protein, but not in the presence of agar. Filtration through paper removes the first vitamine completely and we have a good basis for the idea that the substance from the blood corpuscles belongs to the type of A-vitamine (antirachitic vitamine) which is found naturally associated with fats; or it may also belong to a vitamine type that is easily adsorbed by protein. On the other hand, the vitamine of the tissues belongs perhaps to the B-vitamine type. Blood seems to contain both substances although, in sheep serum, the first one appears to be lacking. Morini (152) grew the gonococcus on gelatin and beer yeast.

The observations of Douglas, Fleming and Colebrook (153) suffice to give us some idea of the conditions under which anaerobic bacteria are best cultivated. Special measures for the exclusion of air are shown not to be necessary, if the bacteria are given the opportunity to provide themselves with local anærobic conditions by the addition of some porous material such as asbestos. It is very important, however, to add to the bouillon culture, potatoes, carrots, cabbages, and grapes, or better still, bran. An alcoholic extract of bran is effective only when large amounts are used.

Tubercle bacilli were cultivated by Sazerac (153a) and by Masucci (153b) on digested casein (aminoids).

In regard to hemophilic bacteria, there is the work of Davis (154). For this type, two factors in particular are of great importance. One is hemoglobin, and the other is a substance which is found in other strange bacteria, as well as in a variety of fresh plant and animal tissues. This second substance is found in *B. influenza* when both strains are cultivated together. The similarity of the factors necessary for the growth of rats with those necessary for the successful growth of hemophilic bacteria has been emphasized by Davis. He explained it by the fact that hemoglobin serves as the source of iron, while the second substance facilitates the assimilation of this element. In a later publication, Davis (155) found that the growth-promoting influence of hemoglobin, white and brown rice, pure wheat flour and wheat bran, was shown by no other kinds of bacteria except by the hemophylic. Germinated rice or wheat kernels exhibit a greater influence than ungerminated. In this case also, the active substance was adsorbable by filter paper and was quite thermo-stable, having resisted a temperature of 100°C. for one to two hours.

The favorable influence of cooked meat on bacterial growth was observed by Wolf and Harris (156), on the *B. histolyticus*, and by Wolf (157) on the vibrion septicus, *B. sporogenes*, *B. welchii* and *B. proteus* (158). Boyer (159) recommended a hydrochloric acid extract of finely divided bones for the cultivation of streptococci, and Otabe (160) suggested a wheat extract instead of meat for staphylococci. We can see from this short list that the addition to the medium of a natural product as a source of vitamine was necessary. For the cultivation of *B. coli*, Dienert and Guillerd (161) prepared a medium as follows: 500 grams of pressed yeast were heated to 50°C., the optimum temperature for the action of endotryptase. In less than 24 hours the autolysis was finished, the yeast having become liquid, yielding 397 cc. of filtrate which was then diluted to two liters. The extract was sterilized for a half hour at 100°C., and finally diluted to 7500 cc. This preparation was used advantageously instead of peptone.

Bunker (162) and particularly L. David and Ferry (163) have made an exact study of the conditions that are necessary in order to obtain a very active toxin from cultures fo *B. diphtheriae*. It was

shown by these investigators that a medium of amino acids and salts did not suffice and that an addition of creatine, creatinine, xanthine and hypoxanthine had no effect. Only when a 0.5 per cent of bouillon was added, did it result in good growth. For the preparation of toxins, the amount of bouillon present must be increased to 10 per cent. Addition of peptone, on the contrary resulted in a slight growth and toxin formation. Toxin is not supposed to be a synthetic product of bacterial cells, but a product of metabolism which makes its appearance only in the presence of certain amino acids and vitamine.

Agulhon and Legroux (164) investigated the influence of vitamine on *B. influenzae* (*B. Pfeiffer*). The growth-accelerating influence of blood, serum and ascitic fluid, could not have been due to protein addition but to the influence of vitamine. The vitamine extract of defibrinated blood was obtained in two ways. By the first, blood was precipitated by about four volumes of absolute alcohol; the precipitate was extracted with the same volume of water, centrifuged and filtered through a Chamberlain filter. By the second procedure, blood was extracted with sodium chloride solution, and the extract heated to 80°C. for 15 minutes. From 5 to 10 per cent of these extracts, added to the usual medium, showed a great influence on the growth of *B. Pfeiffer*. Even a one per cent solution showed a definite effect. It might well be conceived that the vitamines in the cells are present in combination and that alcohol or heating sets them free. They seemed to be insoluble in alcohol, for a cold alcoholic extract was inactive, while one prepared at 80°C. was only slightly active. Acetone extracts, prepared either hot or cold, were inactive. Warming to 80°C. in the presence of alcohol or acetone, with subsequent drying at a lower temperature did not bring about decomposition, since extraction of this powder with the water yielded an active substance. The watery vitamine extract partially lost its activity after heating to 90°C. for 15 minutes. It appears to be more stable when the heating is done in the presence of gelose, although in this case too, it is partially or completely inactivated, depending upon whether the temperature is raised only to 100°or to 120°C. When a totally defibrinated blood was used, it was observed that the vitamines were found in the corpuscles, since the watery extract of the serum gave completely negative results, while the washed blood corpuscles gave positive results. Hemoglobin and blood corpuscle

stroma were negative. Cold extraction of blood corpuscles for 24 hours with a physiological salt solution gave only a weakly active extract. If hemolysis is induced with distilled water before the extraction, then the vitamines are found in solution. In this case, contrary to what has been noted previously in this chapter, the extracts may be filtered through a Chamberlain filter or through paper without loss of activity; this characteristic permits of sterilization without the use of heat. Incidentally, the action of vitamines has been erroneously ascribed to their peculiar physical condition. Legroux and Mesnard (165) extended their experiments to include extracts of kidney, liver and heart. Davis (165a) has pointed out the necessity of two substances for the growth of B. influenzae, one derived from the blood pigment and one of a vitamine nature. The same conclusions were reached by Rivers and Poole (165b), by Thjötta (165c) and by Thjötta and Avery (165d). Thjötta and Avery differentiate between a thermo-stable substance contained in blood, and called "A," and a thermo-labile substance "V" (vitamine) which is found in blood, yeast and vegetable extracts. Crystalline hemoglobin contains only the substance "A."

Finally, there are the vitamine studies of Kligler (166) on bacteria. At first he used autolyzed yeast and found that pneumococci and meningococci do not grow so well on it as on bouillon. In a later work, he (167) investigated the influence of ox heart, goat blood, rabbit and cat tissue and human excreta on gonococcus, meningococcus, pneumococcus, *Streptococcus hemolyticus, B. diphtheriae, B. pertussis* and *B. influenzae.* All of the investigated extracts exerted a favorable action in exact proportion to the amount added. The spleen, liver and kidneys were rich in vitamines, while muscle tissue was only slightly active, and ether extracts were inactive. Mueller (167a) found that streptococcus needed for growth a substance which he regarded as a new amino acid. The streptococcus was cultivated on beef-heart extracts. If this medium was decolorized with "norit" it was unsuitable for growth, but permitted of growth upon the addition of peptone or certain protein hydrolyzates. The growth substance was precipitated from these hydrolyzates with mercuric sulphate, but it was not identical with tryptophane, tyrosine, cystine or histidine. In a subsequent publication the same author (167b) accepted the necessity of three substances for the growth of Streptococcus hemolyticus—one which

remains in the decolorized beef-heart infusion and two others which were found in the hydrolyzate of the commercial casein preparation "aminoids." Of the latter two, the substance "x" was detected in the silver sulphate precipitate and the substance "y" in the filtrate. In collaboration with Freedman (167c), we were able to show that not all pure proteins (hydrolyzed) fulfill the nutritional requirements of Streptococcus hemolyticus. As a rule, purification of these proteins diminished their nutritive value for these bacteria. Other proteins obtained from sources rich in vitamines are with great difficulty, or not all, freed from this growth substance. On the other hand, this growth substance can be adsorbed in a way similar to vitamines, since fuller's earth and norit remove it from autolyzed yeast, hydrolyzed casein and a neutral solution of sodium caseinate. This phenomenon will permit of further investigation. The absence of the growth substance from a number of purified proteins of various sources renders it unlikely that the above growth factor is an integral part of the protein molecule. It is more than likely that the growth substance is similar to that necessary for yeast—and found in the same starting material—the only discrepancy being that those hydrolyzed proteins which act on streptococcus do not act upon yeast. However, this might easily be a matter of inhibition due to the various protein cleavage products. In this connection, Norris (167d) found that casein was a better medium than brewer's yeast for culture.

The many points brought out in this small chapter, touching in particular upon the physical characteristics of the substances which act on bacteria, form, for the time being, the sole criterion for their classification in the group of vitamines. By comparing the characteristics here set forth with the description of the vitamines to follow later, the reader will have the opportunity of making a decision for himself as to which group these substances should be assigned. Looking upon this chapter as a whole, we may conclude that at least some types of bacteria respond to the addition of vitamine with accelerated growth, showing possibly that a small initial quantity of vitamine suffices to render the bacteria independent of any further vitamine additions for a long time. As we shall see in the chapter on the growth of higher plants, bacteria seem to exist entirely independent of any vitamine supply. According to our present knowledge, these are the bacteria which supply the whole organized world

with the vitamines, or with the substances from which they are formed.

All of the above mentioned observations conform preferably with the conception of two or three different vitamines.

FUNGI

Because of the lesser practical significance as compared with bacteria, the question as to the vitamine requirements of fungi received little attention. Lutz (168) made the observation that fungi often show an interruption in growth when the nutritive medium is exhausted. Still, this observation could be attributed to a general exhaustion of nutritive constituents in the medium. According to a paper by Currie (169), *Aspergillus niger* needs no extra addition in order to grow, and yet he states that an extract of old mycellium, or spore bearing portions of the same fungus, brings about quite a definite acceleration in the growth of a new culture of the same fungus.

The only work affording an insight into the vitamine requirements of parasitic and saprophytic fungi is the recent investigation of Willaman (170). He worked with *Sclerotinia cinerea* [(Bon) Schröter], which he sought to cultivate on a solution of purified salts, cane sugar and nitrates. This experiment, and others in which there was also an addition of asparagine and amino acids, turned out negative. The results were different when fruit juices, yeast or extracts of wheat germ were used. The method of procedure was so chosen that the surface of the mycellium was measured in square centimeters, and the number of spores noted. The juices from peaches, plums and apples showed the greatest activity. Besides these, a great number of natural products were tested and found active, at least as regards the growth of this fungus. However, not all of these extracts were equally active on the sporulation. For this purpose, extracts had to be prepared from parts of plants which displayed a very active metabolism. The following could be classed as such: pollen, sporophores of the fungi and the sclerotinia itself, terminal butts of the Phaseolus and sporulated mycellium of Aspergillus. This observation indicates, perhaps, the importance of the two types of vitamine, although Willaman is inclined to assume, in the meantime, the existence of a single vitamine, varying in its effect according to its source of origin. Nevertheless, one must concede

that in the course of this work, the firmly established physical and chemical characteristics of the active substances are best in accord with the conception of a second vitamine.[1] Working with Oidium lactis, Linossier (170a) found that on addition of vitamine, the activation took place only in the first few days.

HIGHER PLANTS

In the first edition of this book, in anticipation of a possible analogy to animal growth, we wrote a small chapter on plant growth without being able to find any supporting experimental evidence; still, it was not necessary to wait for it a long time. In 1909, Clinton (171) made the observation that plant extracts have the power to stimulate the reproduction of certain plants. Haberlandt (172) found that parts of potatoes containing the morphological structure, "Leptom," showed cell division when placed in a nutritive solution. The embryonic structure only was able to undergo cell division without the presence of leptom. This author suspected the presence of a special hormone in leptom corresponding to the internal secretions of animals.

Bottomley (173) was the first investigator to take up this problem seriously. In 1912, he occupied himself with the problem of the ability of certain bacteria to transform the humic acids of peat into water-soluble combinations. He demonstrated at that time that such peat contains all of the chemical combinations necessary for the growth of young tomato plants, buckwheat, radishes, and barley. In the further development of this question, which appeared at about the same time as our earlier vitamine work, Bottomley (174) extracted a substance from bacterized peat with water or alcohol. The addition of phosphotungstic acid to this alcoholic extract yielded a precipitate which, upon decomposition, acted favorably on plant growth. The solution thus obtained was precipitated with silver nitrate, and in the end the growth substance was found present in the same fraction as the vitamine. All active fractions were tested for their influence on the growth of young plants, and then, in order to set forth the results more clearly, the cotyledons, which contain

[1] Willaman defines the vitamines as a class of substances whose presence is necessary for normal metabolism, but which do not contribute to the requirements of the organism as regards inorganic constituents, nitrogenous substances, and energy producing food constituents.

reserve vitamine, were removed after germination without harm to the plant. The results were very significant and showed that the young plants, in a stage in which they could not live by their own process of metabolism, are dependent upon the stock of vitamine which is stored up in the seeds. In mature plants, the vitamines are built up through symbiosis with bacteria, which prepare it synthetically from the organic constituents of the soil and from

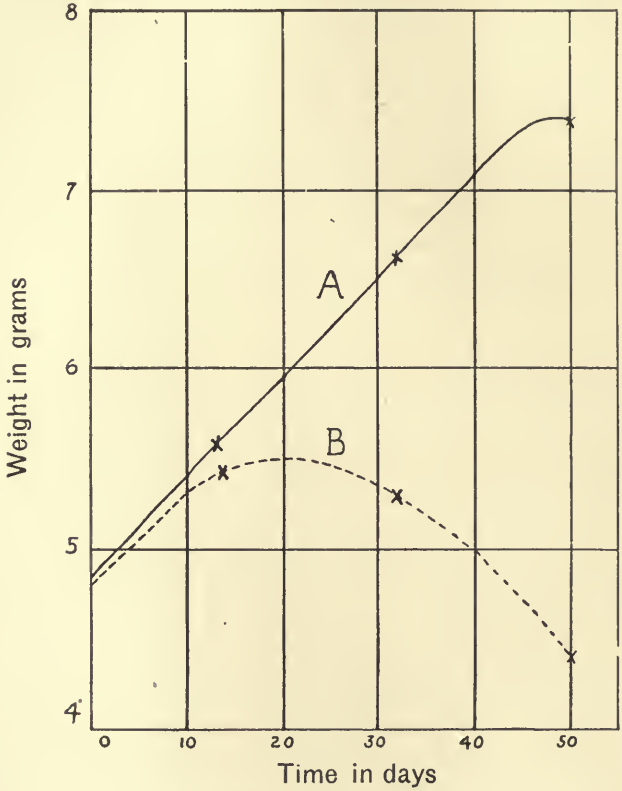

Fig. 4. Plant Growth

A, with vitamine addition; *B*, without vitamine addition (Bottomley)

fertilizers, and carry it to the plants through the roots. In the investigations of Bottomley, the control plants (in the nutritive solution above) underwent a decrease in weight of 10.9 per cent in 50 days, while with the addition of vitamine there was an increase in weight of 59 per cent. The curves appended illustrate what has just been stated. We see here the influence of the silver nitrate fraction

added to the nutritive solution in a dilution of 0.35 in 1,000,000. Bottomley's results were soon confirmed by others (175). The substances influencing plant growth were called "auximones" by Bottomley, who also described a method for their detection (176). This method possesses certain advantages as against the use of higher plants, and consists in an extraction of fermented peat, and precipitation of the resulting extract with phosphotungstic acid. If this extract is added to a culture of nitrifying bacteria and the whole placed in an incubator at 26°C., a scum is formed on the surface after 24 to 36 hours, and no nitrates are found in the solution. On the contrary, without the addition of vitamine, the scum does not appear and the nitrification proceeds rapidly instead. This method seems to be specific for the vitamines, since no such effect has been recorded after the addition of cane sugar, maltose, asparagine, peptone, leucine, tyrosine and hordeine. The microörganism isolated from this scum, similar to the nitrifying and sulphur- and iron-assimilating bacteria, may grow without the addition of organic carbon combinations. Originally, Bottomley was of the opinion that the auximones differed from the vitamines in that the former were heat resistant. We believe, however, that a successful classification cannot be built up on such differentiations. Chittenden (177) repeated these flower-pot experiments in which the ratio of the bacterized peat to the required amount of soil was very high, and therefore was also able to produce an acceleration of growth. When this method of experimentation was applied to greatly extended soil experiments, the results were not so significant; the greatest success was obtained when the rainfall was large. Bottomley (178) himself repeated his first experiment more carefully. For this purpose he used a type of lentil (Lemna minor) that could live in water; this was cultivated on Detmer's nutritive solution, of which the only source of nitrogen was potassium nitrate. This plant could not live only upon inorganic constituents; it gradually became weaker, and presented an abnormal appearance. Bottomley then added to the nutritive solution various extracts prepared from fermented peat. From the results recorded in the table below, only one conclusion may be drawn—that the influence of these extracts was due not only to the presence of certain necessary building stones therein, but also to the presence of vitamines. These experiments were then repeated by Bottomley (179) with other water-plants like Selvinia natans, Azolla filiculoides and Limnobium stoloniferum, with the same results.

The extracts of the fermented peat were examined chemically by Rosenheim (180).[2] To give the reader some conception of the quantities of the active substances used, we shall go into these experiments more in detail: 15 grams of bacterized peat were extracted with 2 liters of water and 500 cc. of this brown solution were diluted to 1250 cc. This extract contained 0.045 per cent of dry substance, 0.034 per cent of organic and 0.011 per cent of inorganic material, and 0.003 per cent of nitrogen. The plants investigated (*Primula malacoides*) received in one dose 0.18 grams of peat, which contained 20 mgm. of organic material and 1.9 mgm. of nitrogen. The plants so treated (figure 5) grew higher, showed leaves of a deep green color, and were also generally larger than the controls, which is especially apparent in the illustration. The substance which could bring about

	VELOCITY OF WEIGHT DOUBLING		UNITS OF TIME NECESSARY FOR DOUBLING OF WEIGHT	
	Number of plants	Original weight	Number of plants	Original weight
Controls	1.0	1.0	100	100
Watery extract of bacterized peat	2.05	3.09	48	32
The same without humus acid	1.91	2.71	52	37
Alcoholic extract of bacterized peat	1.63	2.01	61	50
Phosphotungstic acid precipitate prepared from the last fraction	1.24	1.48	80	67

such a great change must belong to the type of vitamines, according to Rosenheim. From the active extract, it was possible to obtain with phosphotungstic acid a precipitate which was only partly soluble in acetone (like the vitamines, as we shall see later). Rosenheim hoped to extend his experiments to include yeast and milk.

Bottomley (181) investigated the nitrogenous constituents of the peat, and although there was no nucleic acid present, he did find their decomposition products, an uracil-adenine-dinucleotide and the components of guanine-cytosine-dinucleotide.

Appleman (182) believed that potato tubers contained substances which were necessary for the growth of the shoots. It is therefore of importance in planting potatoes, that not too small a piece of the

[2] In this paper, Rosenheim claims priority as to the findings on the importance of vitamines for plants.

tuber be used. He thought that the disease of potatoes common in the United States, and known as "spindling sprout disease," was due to lack of these vitamine-like substances.

Bottomley (183) also investigated the influence of crude nucleic acid derivatives upon the growth of Lemna minor. The action, in

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FIG. 5. GROWTH OF PRIMULA AFTER VITAMINE ADDITION (ROSENHEIM)

this instance, was similar to the activity of a sterilized suspension of *Azotobacter chroococcum*. The combined activity of the two products the suspension and the nucleotides were complementary to each other. Purified adenine-uracil fractions showed no activity.[3] In

[3] Papers published by Jones (184) and also by Stead (185) may be looked upon as reviews.

Bottomley's laboratory, Mockeridge (186) studied the effect of extracts of bacterized peat upon nitrifying bacteria of the soil (*Bacillus radicicola* and *Azotobacter chroococcum*). The results are apparent from the accompanying table, in which the milligrams of fixed nitrogen are given.

1. Nutritive medium alone	2.6
2. Nutritive medium plus watery extract of ordinary peat	1.9
3. Nutritive medium plus watery extract of fermented peat	7.7
4. Alcoholic extract of fermented peat	6.5
5. Phosphotungstic acid precipitate of No. 4	5.6
6. Silver fraction of No. 5	6.5

We may see from these results that the above bacteria, having synthetic ability to assimilate nitrogen from the air, are nevertheless influenced by vitamines. While the vitamine action was favorable to the fixation of nitrogen, the effect upon denitrification was reduced, and the formation of ammonia was uninfluenced. The investigations were then applied to other soil bacteria and it was found, as a rule, that the growth of the nitrifying bacteria is activated by vitamines, while that of the putrefaction, denitrification, and ammonia forming bacteria is not affected. Those bacteria which have the power of decomposing organic matter should in fact have no need for vitamines or else should be able to synthesize them. These two great classes of bacteria, one that begins the nitrogen cycle and the other that ends it, differ from each other entirely in their metabolism if Mockeridge is correct.

It must be especially emphasized that Bottomley and his school always moved further and further away from the importance of the vitamines for plant growth, and ascribed this remarkable action to the nucleic acid derivatives. These conclusions followed in the paths which Schreiner and Skinner (187) and their co-workers laid out for the action of nucleic acid derivatives on plant growth. These investigators isolated from the soil some purine and pyrimidine derivatives and tested their activity. They showed that xanthine could increase the weight of wheat sprouts kept in water about 21 per cent. Similar experiments were made by Macalister (188) with allantoine and by Coppin (189) with other analogous substances. Recently, Miss Mockeridge (190), experimenting with Lemna major, showed that green plants need an addition of certain other substances to continue to live. In this series, the influence of natural

fertilizer was investigated. The results obtained in the fifth week were as follows:

	Average number of plants
1. Control solution	27.0
2. Extract of rotted leaves	64.6
3. Extract of soil	64.4
4. Extract of fermented peat	132.6

It is evident that identical results were obtained with fertilizer and with bacterized peat; indeed, fresh fertilizer was less active than one in which marked bacterial decomposition had set in. Mockeridge then found that nucleic acids and nucleotides predominated in the less active extracts, while in the more active extracts, the amount of free purine and pyrimidine bases was increased. Therefore, the conclusion was made that the effect was brought about not by the nucleic acids themselves, but by their decomposition products. These conclusions are obviously not justified especially since the investigations were not carried out with pure chemical products; consequently the results could be only interpreted to indicate that the substrate acted favorably on plant growth because of some unknown changes that had taken place in the composition of the substrate. Hydrolysis of the afore-mentioned nucleic acids could well be thought of as a secondary occurrence without any physiological significance. It is very likely that the growth-promoting activity of peat and soil extracts is not due to the nucleic acid decomposition products but to the presence of vitamines which are carried down in the fractionation. Should it be established that in the growth of plants, similar substances are involved as in the growth of yeast, then it would be very improbable that they could be associated with purine and pyrimidine derivatives.

Miss Mockeridge's idea of the vitamine cycle, was that the nitrifying bacteria furnish the green plants with the growth-promoting substances which are used in the metabolism of the plants, partly transformed into the vitamines already known to us, and which can then be utilized by the animals.

The investigations of Bottomley and Rosenheim have established that higher plants also need a vitamine of the antiberiberi type in their metabolism, and Bottomley expressed the idea that this vitamine occurred in the plant through symbiosis with certain bacteria. Whether this is the only vitamine that plants need,

remains to be demonstrated. Still, it is apparent that the vitamine of type A, which is found mostly in seeds and green leaves, is formed in the leaves perhaps from primary products which must be furnished to the plants from some outside source. The question, as to whether old plants that already have leaves need extra vitamine in the same degree, must eventually be answered and may perhaps be solved by the use of methods for sterile growth, as proposed by Grafe (191).

Since the entire animal world obtains its vitamine requirements either directly or indirectly from plant food, it is especially important to know the vitamine cycle in its entirety.

CHAPTER II

The Rôle of Vitamines in the Animal Kingdom

Coming to this important chapter, we must first of all satisfy ourselves as to the relative importance of the vitamines in nutrition. In fact, the rôle of the vitamines is placed in the foreground by some investigators, so much so, that the impression prevails that these substances can completely compensate for all dietary deficiencies. Obviously, this is not the case. The requirements of the animal organism for each of the food constituents have been determined chiefly in dogs, and lately in rats; we need only refer the reader to the modern text books on the science of nutrition if he wishes to study the question more thoroughly. We must specifically emphasize, as we did in the first edition, that most of the old and many of the new experiments on this subject were not entirely convincing, since the investigators were either unaware of the existence of the vitamines or else ignored their significance. In the last six years, however, many of the experiments were repeated, bearing in mind the progress that had been made in vitamine research. The conclusions arrived at in these experiments, for example, for rats, are as follows: For complete nutrition, these animals need, above all, a biologically complete protein—a protein of high biological value—one containing all of the necessary amino acids; further, a carbohydrate of the nature of starch, sugar or dextrin; a certain amount of fat, a salt mixture, so chosen that it contains the necessary cations and anions in proper proportion; and finally vitamines A and B.[1] It must be held open for further investigation to determine whether or not rats can get along on a complete protein, a salt mixture and vitamines (without carbohydrate and fat); for some animals, like the dog or cat, which can get along on meat alone, this has already been definitely established. The series of experiments by Spriggs (192) and Maignon

[1] Parsons (191a) recently found that rats do not require a supply of vitamine from outside sources. In spite of the lack of this vitamine in the diet, the organs—spleen, kidney and muscles—of rats so fed contain appreciable amounts of vitamine C when tested on guinea pigs in the form of extracts. Whether this vitamine C originates from the vitamine B of the diet has to be left for future demonstration.

(193), dealing with the harmful effect of an exclusive protein diet on rats, can very likely be attributed to the insufficient amount of vitamines supplied, although it might also have been due to a relative lack of salts. In a special case, Maignon (194) noted that when he fed rats on egg albumin, together with some salts, they developed a disease which he held to be an acute intoxication of the central nervous system, from which the rats died in a few days. It is not improbable that it was some sort of an avitaminosis.

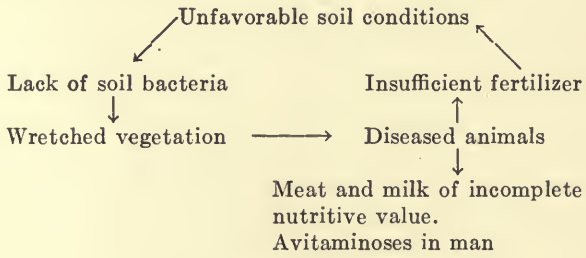
Regarding dietary factors such as nucleins, lipoids, etc., it is quite apparent that they are synthesized in the animal body. It is often specified that for certain animals, and also for man, the animal proteins are preferable to the vegetable proteins. In practice this is quite true, and it may be explained in many different ways. In the first place, when we speak ordinarily of animal and vegetable protein, we have in mind the natural products containing those proteins. Now, there are great fundamental differences between the two types of foods. When we feed an animal protein, like meat, eggs, etc., we give the protein, which is very poor in other dietary components, like fat, and especially carbohydrates, in very concentrated form. With plant products it is different; they contain protein in a very much smaller concentration, accompanied, for the most part, by large amounts of carbohydrates. That this may be of importance, we shall see later. Furthermore, it is possible that the amino acids of animal proteins, because of their close resemblance in composition to those of the body proteins, are better utilized; besides this, they may contain known and perhaps even unknown vitamines carried along during the process of preparation. For example, Osborne, Wakeman and Ferry (l.c. 102) found that it is very difficult to free edestin from vitamine B. As to the relative value of plant and animal protein, we shall go into it more fully in discussing human nutrition.

When we elaborated the vitamine theory in 1912, we stated that these new substances are important or even indispensable to all plants and animals, predicated upon the then available data. The number of facts which we can bring to our support in proof of the above statement has been greatly augmented since the first edition of this book appeared, although not all types of animals have been investigated in this respect. It is quite true that some animals placed upon a vitamine-free or vitamine-poor diet do not remain

in good condition, even though no symptoms of any of the known avitaminoses are apparent. Nevertheless, these exceptions cannot militate against the general significance of the vitamines for the life processes for many reasons. For one, so long as the experimental animals are kept upon an artificial diet made up of purified constituents, they do not live long if extracts of unpurified products are not given. All experiments seeking to demonstrate the contrary have been carried out by feeding a dietary containing either traces of known or unknown vitamines. Then again, it has also been shown that some species of animals can dispense with one or another of the vitamines. This phenomenon may perhaps be explained by the symbiosis of the host with certain bacteria. In the higher animals, these microörganisms are found in the intestinal tract in large amounts and are perhaps able to build up the vitamines from simple substances and convey them to the animal. At the end of this chapter we shall add a short paragraph dealing with the rôle of bacteria in animals, but at this juncture, we shall only remark that no attempt has as yet been made to establish these new viewpoints experimentally.

All these procedures bring us nearer to the point that interests us mostly, namely, accurate knowledge of the subject of human nutrition. At once, we are confronted with questions dealing with economic and agricultural problems. Let us consider the breeding of animals as an example. From everything that has already been mentioned it is apparent that the condition of the soil, which contains quantities of organic and nitrogenous matter, can be brought into relationship with the study of vitamines. The indication for such problems is seen in the publication of W. A. Davis (195) who thought that the poverty of the soil (in Bihar, India) in phosphorus is the cause of an avitaminosis in cows, which is responsible for a smaller yield of milk and is connected with the under-nourishment of children. Similarly, a nervous disease of horses was observed in these villages. In European countries, as well as in the United States, where agriculture and cattle raising are carried out rationally, these conditions are of course to be noted very infrequently. It is different in countries like Australia, Argentine and South Africa, where cattle raising is carried out on a large scale without paying sufficient attention to the care of the soil, due no doubt to the lack of laborers. There we often hear of diseases,

which are not always considered as deficiency diseases, though they might turn out to be such. Because of insufficient irrigation of the fields and periodic dry spells, we see domestic animals, feeding on wretched vegetation for months at a time, dying from diseases which we shall describe more in detail further along in the chapter. Here, we are perhaps dealing with conditions which follow each other like the individual links of a chain. As a result of the unfavorable condition of the soil and the paucity of soil bacteria, the vegetation suffers. The animals which must feed on this vegetation either become sick, or else are not in perfect health, though no unusual symptoms may be apparent. Subsequently, their offspring and the children drinking the milk of such animals are naturally poorly nourished; the same is true of grown people who use the meat of such animals. Indeed, these conditions could as well prevail in European countries where, because of the war, the number of cattle has decreased, leading to the exhaustion of natural fertilizer, which could not be replaced by artificial fertilizer because of the difficulty of obtaining it. The diagram, illustrative of the relationship between the above mentioned factors, is shown below.



LIFE WITHOUT BACTERIA

As we have stated several times previously, the incomplete knowledge of the rôle of the bacterial flora in animals is the cause of a great gap in the conception of the general significance of the vitamines. We know, or at least we suppose, that some bacteria are independent in their metabolism of an outside addition of vitamine, and since we know that a great number of bacteria live in the intestinal canal, either in symbiosis or else as a parasite, we can easily imagine that their functions may have something to do with supplying the host with vitamines.

Long before the introduction of the vitamine theory in the study of nutrition, Schottelius (196) sought to solve the problem as to whether it was possible to raise animals in a sterile state, and to keep them alive for a long time. After overcoming great experimental difficulties, among which was the construction of specially designed apparatus, he was finally able to raise and keep young chickens under sterile conditions. The animals, however, soon became cachectic and died. Using similar methods, Belonowsky (197) was no more fortunate. The problem was then undertaken with lower and higher animals by various workers. Of the lower animals, flies were chiefly used. In this connection, there are the experiments of Bogdanow (198) who attempted to raise larvæ of meat flies (Calliphora vomitoria) in a sterile condition. He proceeded as follows: the eggs were sterilized with sublimate and the larvæ cultivated upon sterile meat. They grew very slowly, and showed no metamorphosis. Since they did not survive, Bogdanow concluded that bacteria are necessary for this type of insect. In a number of experiments, Delcourt and Guyénot (199), and also Guyénot himself, sought to reach the goal by the use of a different method. They attempted to obtain aseptic larvæ through successive cultures on an acid medium. They noted that flies could live on yeast and, after several generations, also on sterile potato. Larvæ grew well on peptone and salt, but showed no metamorphosis. Wollmann (200), following the method of Bogdanow, carried these investigations further, and came to the same conclusions as the latter.

A number of animals, like the scorpion and winter lizard, are supposed to have sterile intestines and hence are specially suitable for such experiments. Mme. Metchnikoff (201) tried to raise frogs' larvæ in a sterile state but without success. Moro (202) had already made similar experiments with larvæ of the alliaceous toad. The larvæ could be kept alive for 35 days, but their growth was much less favorable than that of the controls. Since all these experiments appeared to be rather indefinite (at least for the pre-vitamine period), Cohendy (203) undertook the experiments of Schottelius with chickens. For this purpose, he built a very ingenious apparatus, in which the food, together with the whole paraphernalia, was sterilized at 115°C. for 25 minutes, or for 1½ hours at 118°C. Cohendy reported that he had obtained good results with this arrangement, and came to the conclusion that life is quite possible in the absence

of bacteria. Upon reading through his work, one receives the impression that although the animals ate more food than the controls, they weighed much less, and soon died. The longest duration of life was 40 days (204). Schottelius (205), criticising the above work, expressed the idea that Cohendy's experiments showed, on the contrary, that the intestinal bacteria were useful, or even indispensable. Such experiments were likewise carried out with mammals. Küster (206), working with goats, was able to maintain young animals in a sterile condition for 35 days, during which time no difference from the controls was noted. It is not impossible that these experiments did not last long enough. Charrin and Guillemonat (207) carried out similar experiments on guinea pigs, while Kianizin (208) used guinea pigs and rabbits. The latter made his animals breathe sterilized air and eat sterilized food. The poor results of this regime were noticeable in from 4 to 9 days; the animals suffered from weakness and died soon thereafter. Nutall and Thierfelder (209) conducted the well known experiments on guinea pigs in which these animals were kept free from bacteria and symptoms of sickness and even showed a gain in weight. In this case, too, the experiments were of rather short duration.

These experiments required corroboration from the viewpoint of progress in vitamine research. This view is held likewise by Weill and Mouriquand (210) and Schaeffer (211). It must be pointed out that while all the investigators working on the problem of bacteria-free existence thought that they were dealing with only a single unknown factor (the rôle of bacteria), there were really two to be considered. During the process of food sterilization, the vitamines—according to the choice of food and the conditions of temperature—were more or less affected by the heat, and probably destroyed. It is obvious, therefore, that the problem of a germ-free existence was much more complicated than was imagined by the above mentioned investigators. From the facts known at present, we may conclude that in some animals, in which it is not possible to produce an avitaminosis by means of a diet either poor or lacking in vitamines, the bacterial intestinal flora possibly assume the task of preparing the necessary substances for the host. To solve the question beyond all doubt, it is necessary to proceed so, that in the first place we arrange for a bacteria-free growth; if this should fail, the animals may perhaps be saved through the addition of vita-

mine. It may then be possible to experiment with the pure cultures of the intestinal bacteria indigenous to the particular species, to produce a normal dietetic condition without the addition of vitamine. Only then shall we be in a position to state that vitamines are necessary for the existence of the entire animal kingdom and eventually for the whole organized world.[2] Sometimes, however, a poor idea has some truth in it, and this seems to be the case with Portier's hypothesis. It is possible, as we have already said, that certain animals and plants obtain their vitamines through a symbiosis with bacteria. This idea was tested experimentally by Portier and Randoin (217). They made the observation that rabbits fed on sterilized cabbage and carrots developed an avitaminosis, while rabbits which received an addition of the excrement of another rabbit on the same diet remained normal; still, it is not quite clear to us why rabbits kept on the same diet should possess a different intestinal flora than those receiving an addition of feces.

It appears that these experiments, serving to demonstrate the importance of the symbiotic intestinal bacteria, could be much better explained on the basis of the vitamine content of the feces.

PROTOZOA

Investigations on protozoa were carried out chiefly with paramecia. Lund (218) has recently shown that he grew these single-cell organisms on yeast extract, but the details of the experiment have not yet been published. Calkins and Eddy (219) worked with Paramecium aurelia kept on two different nutritive media—hay infusion and cooked flour. As a source of vitamine, a preparation of vitamine B of the pancreas was used. The number of daughter cells varied within the limits of experimental error, and therefore it was concluded that the vitamine exerted no influence on this type of animal. Flather (220) obtained somewhat dissimilar results from experiments in which pure cultures of species of paramecia were maintained always under the same experimental conditions; in every experiment,

[2] Portier (212) has recently formulated a hypothesis in which he assumes that in certain plant and animal organs, there are heat resisting bacteria (called symbiotes) which behave biochemically like vitamines (Bierry and Portier (213). Just now, we shall not discuss this theory itself for it appears to be lacking a real basis, and besides it has already been made the subject of an earnest critique by Delage (214), Lumière (215) and Ranc (216).

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three cells were grown in two drops of water, with the addition of one drop of a 1 per cent watery solution of polished or unpolished rice, or malted milk. The division factor was taken as a measure of cell activity and the first 24 hours were disregarded, to give the cells the opportunity of adjusting themselves to the new conditions. The following values were obtained: for white rice 0.34, for unpolished rice 0.58, for malted milk 0.84. It was then possible, by the addition of malted milk, to make the nutritive value of the white rice approximate that of the malted milk Other experiments were made with various concentrations of malted milk and also with orange juice, with the result that an addition of malted milk in higher concentrations was of no further advantage, while orange juice showed a very slight activity Addition of orange juice to white rice was followed by better results than when added to unpolished rice, and with orange juice alone, no paramecia cells could live after six days. In these experiments, the influence of vitamines is easily recognized; in fact, Flather was undoubtedly dealing in this case, not with one vitamine but with the combined influence of the mixture of vitamines.

Chambers (221) carried out similar experiments which demonstrated the slight effect of potato extract; this displayed some influence only if the nutritive solution did not contain sufficient dietary constituents; the same was true of malted milk. Peters (222) cultivated Colpidium colpoda on a nutritive solution which contained, besides salts, a mixture of some amino acids and in some cases only one amino acid, tryptophane. The mixture of amino acids was more active than the tryptophane alone. The first divisions took place very slowly, though it was possible to keep the cultures alive for three months through sub-culture. Peters explicitly emphasized that he could find no symbiotic microörganism. He stated that in certain stages of development, a vitamine-like substance seems to play a definite rôle. In all these experiments, having for the purpose the study of the influence of the vitamines, it is important that the food mixture used should be proved to be vitamine-free. Abderhalden and Köhler (l.c. 127) investigated the action of yeast extracts on flagellates (colpoda) with positive findings.

When we leave the protozoa, we find a dearth of available work on other kinds of animals, although it should not be thought that such work does not exist. This is specially true of the polycellular

animals investigated at various biological experimental stations, where observations which would be of interest to us have undoubtedly been made on the modes of nutrition of many insects. Unfortunately, these data were unavailable to us.

METAZOA

Coming to the polycellular animals, we shall first describe a series of experiments undertaken for some other purposes. They deal with the influence of lecithin upon growth and were carried out by Goldfarb (223). In this respect, he investigated a whole series of animals, and also tadpoles and eggs of the starfish. At first, he stated emphatically that lecithin had a definite action, but in a later work (224), he admitted that it was inactive. This is apparently associated with a vitamine impurity, whose presence may vary with the mode of preparation of lecithin.

It may not be amiss to say a few words here on Carrel's tissue culture in vitro, which in the future may perhaps be of help in advancing the vitamine problem. These tissue cultures may be thought of as polycellular organisms.

The growth of tissue in vitro

Most of the experiments of Carrel were made at a time when the vitamines were either unknown or given very little consideration; otherwise it might have been possible to choose still better conditions for tissue culture. We must admit, however, that Carrel practically foresaw the future trend of work, in that he chose the natural juices of the animal organism as the nutritive solutions. Inasmuch as the author has little practical knowledge of this method, he is naturally not in a position to judge whether or not Carrel would have obtained even better results if he had tested his nutritive solutions for the presence of all the necessary elements for nutrition. Because of the great interest that experimental surgery of the future may have in this method, it is very important to obtain the greatest success. The tissue fragments used in these experiments, are removed from their normal environment and must then lead an independent existence. For this purpose, they need all of the dietary constituents (also vitamines) designated as essential by the modern science of

nutrition. By means of frequent sub-cultures, Carrel prevented the nutritive substances from becoming exhausted. That this had to be resorted to frequently in order to keep a fragment of tissue alive for a long while, shows perhaps that the conditions for growth could have been better chosen.

In this respect, the method may be applicable to a new field of work. We have in mind particularly Carrel's antiseptic method of treating wounds, which came into use during the war. It is apparent that the organism suffering from shock and other complications could not be in the best nutritive condition; and in the wounded tissue itself, through the disturbance of the blood circulation, conditions could not be the most favorable. For these reasons, it would be of interest to apply to the treatment of the wound, those facts that have been learned in the study of the composition of nutritive fluids best suited for the tissues. Practically, this could be brought about by the substitution of the antiseptic solution by a nutritive solution from time to time, with the possibility that the wounded tissue could be nourished and the process of healing hastened.

The method of Carrel (225) is as follows: Under appropriate conditions, tissue fragments, and pieces of tumors too, may be grown for more than four months, during which the tissue shows growth and peripheral expansion. The tissue (226), antiseptically prepared, is placed in a plasma diluted with $\frac{1}{4}$ to $\frac{2}{3}$ of its volume of distilled water. For this purpose, it is best to use the plasma of the animal under investigation or that of a homologous animal. In from 3 to 4 days, the tissue culture (Carrel (227), Carrel and Burrows (228)) is placed for a few minutes in Ringer's solution and then transferred to a fresh plasma. Some tissue cultures have been kept alive for four and a half months, having been transferred as above 48 times. A fragment of heart continued to beat after 140 days. Only embryonic tissues and tumors could grow in an artificial medium (Locke's solution, agar and bouillon). In a later work, Carrel (229) investigated in greater detail the influence of tissue extracts and body fluids (as did Walton (230) too), and found that they increased the growth of connective tissue in particular from 3 to 40 times. This was the case especially with embryonic spleen extracts, and it was considerably smaller with greater dilutions. It was true only of the same type of animal, and the action of the extracts was weaker on heating to 56°C. and entirely dissipated at 70°C. The active substance

could be filtered through a Berkefeld filter, but not through a Chamberland filter. Regarding the foregoing, it appears likely that a new field for vitamine research is opened up.

INSECTS

We have to deal here with the very interesting studies of Jacques Loeb (231). He demonstrated that a type of fly (Drosophila) could attain the larva stage upon a solution of cane sugar and salts, together with the addition of filter paper. The larvæ grew quickly, especially on the addition of alanine or an ammonium salt. In this particular instance, no attention was paid to the influence of the bacteria on the substrate. Five successive generations of the banana fly (232) were grown on a solution of glucose, cane sugar, ammonium tartrate, citric acid, di-potassium phosphate and magnesium sulphate. As Loeb himself observed, it was not quite certain that the solution was free of bacteria; the presence of yeast in particular was not excluded. In these experiments, the flies themselves were not sterile and therefore Loeb and Northrop (233) started experiments with flies hatched from eggs sterilized by a sublimate solution. Twelve sterile generations were cultivated in this manner, carefully protected from every possibility of contamination with yeast growth. The nutritive solution used was composed of 450 grams baker's yeast and 50 grams citric acid (used for the purpose of decreasing the danger of infection during the various manipulations) in a liter of water. In a great number of experiments, the investigators showed that on another medium, for instance, filter paper and cane sugar, with the addition of salts, and likewise with the addition of casein, edestin, egg albumin, milk, or a mixture of amino acids, the larvæ attained normal size but could not be brought to the stage of metamorphosis. Sterile flies, grown on sterile bananas or potatoes, showed no sexual development. The results with yeast as substrate were different; here, all the substances are manifestly at hand, which are indispensable for the growth and development of flies. Butter, nucleic acids, thymus and thyroid extracts were without influence, and the authors believed that these substances are different from those which are of importance for pigeons, rats and other warm-blooded animals. This view must naturally undergo a slight modification, since there is obviously no reason to differentiate between flies and warm-blooded species. The

yeast substance was heat-resistant but lost some of its activity on treatment with cold or hot alcohol. Drosophila is a monophageous animal and yeast seems to be the only adequate food for it. These flies can live in any nutritive solution that is suitable for the growth of yeast, and the medium is usually infected with this organism. Loeb and Northrop rightly observed that in considering the synthetic abilities of the higher animals, one must not lose sight of a possible coöperation of the intestinal bacteria. Loeb and Northrop (234) and also Northrop (235) showed by means of a specially planned series of experiments that the developmental stages of flies (except imago) can be prolonged from 8 to 17 days at will. They believed that the three stages of metamorphosis could be regulated by the formation and disappearance of three different substances.

In a later work, Northrop (236) investigated the rôle of yeast in the nutrition of flies. He found that the number of flies that could be developed on a certain quantity of yeast could be increased by the addition of bananas, casein or sugar. The rate of growth on a combination of yeast and bananas is greater than on yeast alone. In mixtures containing a small amount of yeast, growth proceeds more slowly, and in a dilution of 1 to 128, it becomes entirely abnormal. Kidney, liver, dog pancreas, mouse liver, and bodies of flies themselves were suitable for the growth of the larvæ, while sterilized spleen, heart muscle, suprarenal glands, thyroid and blood of the dog were unsuitable; the addition of muscle tissue, testicles and sterilized thymus gland of the dog, rabbit and calf, resulted in the development of some chrysalis, but growth proceeded slowly and the flies appeared dwarfed. Robertson's tethelin (lipoid from the hypophysis) was inactive.

Guyénot (237) attributed to the vitamines some part in the development of fly larvae of the *Drosophila ampelophila*. Baumberger (238) investigated some species of flies and came to the conclusion that *Drosphila melanogaster* can live on fermenting fruits and also on yeast protein. A combination of yeast, nucleo-proteids, sugar and a salt mixture provides a suitable food for larvae. Other kinds of flies, like *Musca domestica*, *Desmotopa*, *Sciaria* and *Tyroglypha*, can all thrive on microörganisms.

From this chapter, we see, especially from the work of Loeb, that flies need vitamines for their development, and that vitamine B plays the most important part.

FISH

As we progress in the animal series, we do not meet with any vitamine experiments—at least to our knowledge—till we come to fish, the single great group typical of the vertebrates, of which we have no knowledge in this respect. There is only the work of Morgulis (239) which might be considered to have some slight relationship to our subject. He fed ox heart to fish and found that it is better assimilated when cooked, while ox liver had no particular peculiarities. Almy and Robinson (240), comparing dry and fresh fish food, found that with the latter there was less mortality and a more definite weight increase.

AMPHIBIA

Emmett and Allen (241) used frog larvae in their vitamine studies, but the fact that the larvae consumed each other complicated the experimental work somewhat. The larvae reacted poorly towards a greater addition of fat, and developed better on a diet containing 5 per cent fat than on one containing 28 per cent. Both vitamines, A and B, appeared to be necessary, although the latter seemed more important. The variation of protein content from 10 to 30 per cent was of no special significance, although the optimum growth was obtained with higher concentrations. Lactalbumin or a mixture of beef and oats was superior to corn gluten, indicating the importance of the protein used. The growth of the hind legs depends upon the addition of vitamine, according to the findings of Emmett, Allen and Sturtevant (242). For complete metamorphosis, the addition of iodine also appears to be necessary.

Harden and Zilva (243) investigated both larvae and adult frogs. With tadpoles kept in water to which was added fresh meat, a normal development was noted, while an experimental diet made up of 20 per cent casein, 75 per cent starch, 5 per cent salts with the addition of butter, yeast extract, and orange juice, did not permit of normal development, since only two out of six animals attained full growth. According to this a better experimental diet had to be prepared. With adult frogs, another difficulty presented itself in the development of a fungus-like disease. On a diet free from vitamine B, some of the animals stretched out their legs, before death, and one suffered from convulsions. Without A- and B-vitamines, seven frogs died in about four months. Out of five animals on a diet containing the

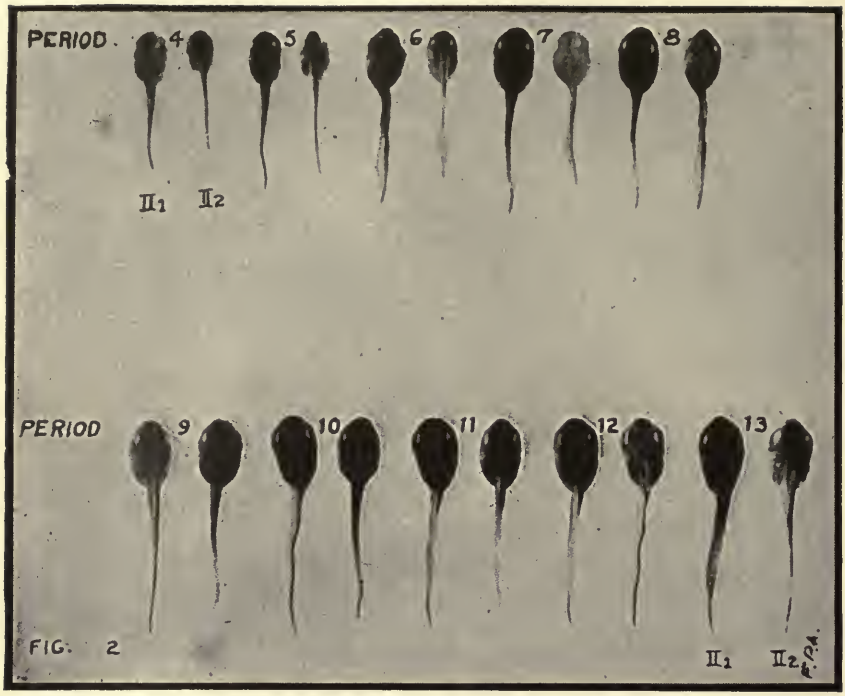


FIG. 6. GROWTH OF FROG LARVAE

Diet II_1 contained A- and B-vitamines, while diet II_2 was lacking in B-vitamine (Emmett–Allen).

FIG. 7. GROWTH OF FROG LARVAE

Diet II_1a was complete while diet VI_2 was deficient in vitamines (Emmett-Allen).

above two vitamines, with or without vitamine C, only one died. Out of six animals getting A-vitamine but not C-vitamine, all died in about 155 days. Out of five animals receiving vitamine B, with or without C, four survived for 250 days.

Accordingly, B-vitamine is necessary for the maintenance of grown frogs, although the symptoms were noticeable only after six months, while the C-vitamine appears to exert no influence on the animals.

BIRDS

Chickens

As stated in the historical part, Eijkman (l.c. 50) and Grijns (l.c. 52) were the first to experiment with chickens. It was particularly noted that Eijkman, by feeding chickens with the food discarded by a beriberi hospital, observed that they developed a disease which he called "Polyneuritis gallinarum." Eijkman soon found that other foodstuffs, like sago and starch, could also bring on this disease.

Normal nutrition of chickens. We shall first consider the normal dietary requirements of the chicken. One who has tried to raise young chickens in the laboratory knows the great difficulties that have to be dealt with. The first attempt in this direction was made by the author (244) who investigated the influence of various kinds of foods on the growth of experimental tumors. In spite of the great eagerness with which the chickens ate the food, growth ceased and they developed a characteristic disease of the legs; the toes lost their vigor and were bent. Very often it was noticeable that the beak was not closed properly, and the animals died because of their inability to pick up food. The eyes were frequently closed, and the chickens suffered from a disease which gradually led to complete blindness, and which would have been regarded to-day as ophthalmia. A change in the diet was not enough to better conditions since the animals were already receiving quite a variety of food. An attempt was made to feed the chickens with live grub-worms, and for a time the floors of the cages were covered with grass and earth, which was frequently renewed. We believed then that the wooden floors had some pathological effect on the legs of the animals. However, all these measures were of no avail and the birds became sick, showing symptoms which we then attributed to rickets. On this basis, we used cod liver oil as a therapeutic measure. As the figures below indicate, a young chicken could maintain itself for eight months on

unpolished rice and cod liver oil (Funk and Macallum 245). The animal was normally built but weighed only 160 grams while a chicken of this age should weigh about two kilos. No symptoms that could have been regarded as rachitic were noted.

The animal had a large beak and excessively long feathers for his size; all secondary sexual indications were lacking and it chirped like a four weeks old chick.

On white rice, the young chickens died (l.c. 244) after about two weeks, while on unpolished rice it was possible to keep them alive for more than 5 weeks. When the young chickens were fed Spratt's chicken food, the weight increased more than twice, while on unpolished rice for 4 weeks, there was no growth at all. These experiments were conducted with 15 days old chickens and were later repeated, with the same results (Funk 246). In all exact feeding experiments with chickens, it is necessary, particularly during the summer, to cover the cages with muslin, otherwise they eat so many flies that the results may be misleading. We have often observed that a chicken which grew in spite of a deficient diet stopped growing promptly as soon as the cage was covered.

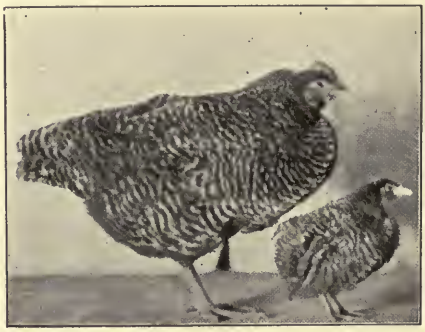


Fig. 8. Seven Months Old Chickens

Normal diet and a diet of unpolished rice and cod liver oil. (Funk-Macallum). Left—2500 grams; right—160 grams.

Drummond (247)[3] carried our experiments further and criticized our results rather sharply. He found that the possibility of raising

[3] After my departure from the London Cancer Hospital Research Institute in 1915, my former assistant, J. C. Drummond, continued a great number of experiments started by me, without communicating with me, and without stating in his publications that the ideas had originated with me.

chickens in the laboratory to full growth depended upon the age of the chickens used. He observed that the animals often failed to grow on a normal diet, and he accordingly believed that this suppression of growth had nothing to do with the vitamines, although his results with white and with red (unpolished) rice, as compared with a normal diet, were identical with ours. Drummond then tried to improve the condition of the animals by giving them earth, sand and wood chips, but without success. We showed later (248) that it was possible to decrease the mortality of the animals and to improve their general condition, by adding animal charcoal to the diet and substituting milk for the drinking water.

Buckner, Nollau and Kastle (249) demonstrated that young chickens did not grow well on a diet poor in lysine, as compared to one rich in lysine; the addition of butter was without effect. It is not improbable that the diet rich in lysine possessed altogether better nutritive value. Similar results with lysine were obtained by Osborne and Mendel (250). In a later investigation, they (251) occupied themselves with the problem of raising chickens in the laboratory. They stated, in accord with our results, and in opposition to those of Drummond, that it is quite possible to raise young chickens in cages. On a diet containing butter as a source of vitamine A, and an addition of filter paper, it was possible to raise some several weeks old animals to normal size. Although most of the animals suffered from weakness in the legs, the investigators believed that as soon as all conditions for the growth of chickens were known, it would be possible to conduct growth experiments similar to those with rats. In these experiments, no green food was given and the diet was thought to be completely carrotinoids-free, which was denied by Palmer. Hart, Halpin and Steenbock (252) were of the opinion that the disease of chickens mentioned above, designated "leg weakness," could not be considered as an avitaminosis. Practical chicken breeders attribute this disease to the close confinement, lack of green food and over-eating. The above workers explained the condition as being due to constipation and stated that the green food, because of its vitamine content and cellulose, might have had a beneficial influence on the constipation. They found indeed that green food is not necessary, and that the same results could be obtained by giving filter paper, dirt or animal charcoal instead; it was necessary to mix these substances intimately with the diet. These additions

served not only as roughage, preventing constipation, but also to dilute the diet and make it more easily utilized. In most cases, the diet consisted of 25 per cent casein, 38 per cent dextrin, 15 per cent butter, 5 per cent salt mixture, 2 per cent agar and 15 per cent dried yeast. With less casein, butter, yeast and salts, and more dextrin, in making up a diet which is very good for rats, no growth was observed in chickens. The experiments lasted from three to four months and it still remains to be seen whether with this régime the experiments can be duplicated, for only then can we regard the problem of the synthetic nutrition of chickens as solved. Filter paper seemed to be the best substance to use for roughage, although an addition of 10 per cent animal charcoal was also effective.

Palmer (253) carried out a series of experiments in which he tried to raise animals, and particularly chickens, on a diet completely free from plant pigments. The experiments are of special interest because of their relationship with the antirachitic vitamine, to be referred to later. Palmer found that some of the animal pigments belonging to the carrotinoid group, have their origin in the vegetable kingdom. The species having a colored adipose tissue obtain this pigment from the blood, in which it is easy to demonstrate its presence. In a series of feeding experiments with chickens, Palmer and Kempster (254) showed that in some foodstuffs a certain relationship exists between the carrotinoid and the vitamine A content; they demonstrated, however, that this relationship is only apparent and that the pigments, per se, play no part in metabolism. They also experienced considerable difficulty in raising the chickens in a closed room, and further, in finding a diet free from pigments. In the end, they chose a diet composed of white corn, bran from the white corn, bleached flour, centrifuged milk, and bone meal. The chickens used were white Leghorns and weighed from 700 to 750 grams at the start; the colored parts of the body, like the beak, and the feather quills, were only weakly colored; the mortality was quite high. After six months, there were only five out of eleven chickens still alive which had doubled their weight and begun to lay eggs. The eggs were not completely pigment-free, but were nevertheless only weakly colored. Very young chickens could not be raised on this diet. Better results were obtained when filter-paper was added, according to the method of Osborne and Mendel.

However, instead of using butter, which is not carrotin free, pig liver was used as a source of antirachitic vitamine. Sixty chicks were placed on this diet, upon which they grew well for about 6 weeks, but then began to lose ground. At this stage of the experiment, young pigs' liver was given with the result that an immediate improvement was seen. The animals, which were 3 months old, were of normal size and weight and received during the summer an addition of pigment-free vegetables, such as white onions. Palmer and Kempster concluded that young chickens could live without carrotinoids, if the diet contained sufficient vitamines. They believed also that the possibility of discovering the nature of vitamine A by an investigation of the carrotinoids was without foundation in fact. The animals fed on a carrotinoid-free diet laid eggs after 6 months, and yet the chickens in the second generation were few in number and rather puny in appearance.

Hart, Halpin and McCollum (255) and Hart, Halpin and Steenbock (256) studied the nutritive requirements of chickens. For their experiments, they used half-grown animals fed on a mixture of corn, corn gluten and calcium carbonate, and also wheat, wheat gluten and calcium carbonate. The birds grew well and were able to lay fertile eggs. These findings stood out in marked contrast to the results obtained with rats and pigs, and showed satisfactorily that the nutritive needs of chickens and some mammals were totally dissimilar. In chickens, an addition of salts, casein and butter were without the slightest influence, while the protein concentrates acted favorably. These results might be attributed either to the influence of protein concentration or to the addition of unknown factors, which are not present in cereals and casein, and which exert a favorable influence on egg production. The experiments with younger animals (l.c. 256) resulted somewhat differently. In this case, corn was a sufficient source of B- and A-vitamine, but not so with wheat, upon which the animals lived only three months. It was only when salts, casein and butter were added that the diet appeared to be complete. In a similar manner, Harney (257) was able to show that for the production of eggs, the use of plant foodstuffs, in spite of the high protein content, could not be compared to that of animal food, and besides this, Kaupp (258) held that an addition of skimmed milk could influence the production of eggs favorably.

In accordance with these experiments, we must recognize the importance of two vitamines, A and B, for the growth of young chickens. For the grown animal, however, the importance of vitamine A had not been set forth so clearly. The recognition of these related facts is important for the proper consideration of chicken beriberi,[4] a disease arising largely through feeding with white rice. McCollum and Davis (259) were able to show that this kind of rice lacks, besides vitamine B, protein of high biological value, some salts, and vitamine A. Whether these results, obtained with rats, are applicable to other animals without further work, is at best questionable; yet we must consider the possibility that when we try to cure animals, fed with white rice, by adding vitamine B, the indefinite therapeutic results sometimes obtained might be due partially to the lack of other dietary constituents.

In conclusion, we shall mention the work of Houlbert (260) who kept chickens on white rice, wheat and barley, heated in an autoclave to render them poor in vitamines; twice a week, cod liver oil was added. After 40 days, it was found that the division of cells in the sexual and hematopoetic organs had come to an end. However, if the missing vitamine was replaced in the diet, the glands resumed normal development.

Chicken beriberi. In relation to the experiments of Eijkman and Grijns, chicken beriberi, besides being brought on by white rice, may arise from feeding white bread (Hill and Flack 261), an observation also made by Ohler (262) and by Weill and Mouriquand (263). Ohler also produced the disease by feeding hominy, but not that from whole corn. Based upon our experiments, Wellman, Bass and Eustis (264) used cane sugar and corn starch; Wellman and Bass (265) also used macaroni. Naturally, these experiments are only of historical interest since they showed that the etiology of beriberi is not related only to the rice consumption. We have known for a long time that all food poor in B-vitamine can give rise to beriberi.

In the work of Vedder and Clark (266), there is an excellent description of chicken berberi. The first symptoms, on an exclusive

[4] In agreement with some others, in the light of the facts established in this field of work, I have dropped the designation "Polyneuritis gallinarum," since according to the newest developments, it has nothing to do with neuritis, but perhaps with a general change in almost all of the tissues. For a long time, I have used the term "experimental beriberi of animals" for this condition.

diet of white rice, appear in from 20 to 30 days. At first there is paralysis of the extensors of the legs; the bird sits on a flexed tarso metatarsal joint. Paralysis soon extends to the wings, nape of the neck and the entire musculature. The animal then lies motionless on its side; a deep prostration appears frequently on the second or third day after the onset of paralysis—at the latest, in one week—and is followed by death in all cases; the whole course of the disease is run in a very short time. From the characteristic symptoms of experimental beriberi, the following should be noted particularly:

The crest becomes bluish red very soon, and this appearance may be considered as one of the initial symptoms.

FIG. 9. AVIAN BERIBERI

First day symptoms (Fraser-Stanton)

Paralysis of the wing musculature belongs to the later symptoms—the wings then hang loose down to the ground.

Spasticity seldom appears in the initial stages of the disease, but more often in the later stages. We notice here the retraction of the head to the back; sometimes there is seen the spastic walk on tiptoes with stiff knees. Dysphagia belongs to the early symptoms; water given to the bird flows out again from the beak; on attempting to feed forcibly, the pigeon chokes. According to Vedder and Clark, the loss of weight is constant at about 20 per cent of the initial weight.

Progress of the disease. The above named investigators describe the progress of the disease in the following manner: In acute cases, with prostration and great loss of weight, all the symptoms are

apparent in 24 hours. The bird lies on one side, shows dyspnoea and cyanosis and dies in a few hours. The second type is more chronic; in these cases we see paralysis of the legs, but good general condition. The appetite remains good, the loss in weight is insignificant; the

FIG. 10. AVIAN BERIBERI

First day symptoms (Fraser-Stanton)

crest remains red; in this condition, the chicken may live for weeks. According to our personal experience, the second type is not so suitable for curative experiments.

Segawa (267) also described two types of beriberi—one form which he regarded as a pure polyneuritis, and another, possessing more of

the characteristics of starvation, and expressed by a distinct aversion to rice. In 66 per cent of all cases, both types occurred together. In Segawa's experiments, some animals remained in good condition for about 219 days; this may perhaps have been due to the possibility that the animals had eaten traces of other food, such as flies and vermin. He erroneously considered the disease as a real polyneuritis. Tasawa (268) observed that the marked emaciation and starvation could be avoided by an addition of egg-yolk or cooked meat, whereupon only clear beriberi symptoms are noted.

As we shall see later in pigeons, fatigue plays a big part in the development of acute symptoms. In accord with this, Hulshoff Pol

FIG. 11. AVIAN BERIBERI

Second day symptoms (Fraser-Stanton)

(269) found that forced feeding accelerates the acute symptoms, and Williams and Johnston (270) found that the same effect could be hastened by increased temperature and exercise.

Pigeons

The problem as to the normal food requirements of pigeons appears to be much simpler than that of chickens. Since pigeons may be raised in the laboratory with little difficulty and since they do not eat vermin and flies, the feeding experiments may be conducted with greater exactness. Young pigeons, after they have been hatched, are fed alternately by their parents, so that it is possible, through the

administration of a special diet to the latter, to study its effect upon the young from the very beginning.

We have personally kept grown pigeons on an exclusive corn diet for more than a year, during whch time they were not only in splendid health but reproduced normally.[5] It cannot be emphasized sufficiently, *that whole corn provides sufficient nutrition for the maintenance and growth of pigeons*, which indicates that corn is quite sufficient for at least one species. This is of particular importance for our later conception of the etiology of pellagra, which has recently been attributed to the deficiency of corn proteins. That corn is a complete food for pigeons has been shown by other workers. Voegtlin and Myers (271), reported that they maintained pigeons in good health on an exclusive diet of corn for at least four months. In another series of experiments, the same workers (272) investigated the conditions of growth in young pigeons which attained normal size in 40 days, after having been fed by their parents who had been given a diet of whole corn or whole wheat, with the addition of a calcium salt. The same result was obtained when the diet was composed of white bread, with the addition of vitamine B and calcium salts; and also wheat starch, casein, A- and B-vitamine, and calcium salts. Because of the possible interest in the study of the growth of young pigeons, there is appended a diagram illustrating normal growth as compared with growth on corn, taken from the work of Voegtlin and Myers.

It is shown that young pigeons require two kinds of vitamines, namely, A- and B-vitamines, and can get along very well in the entire absence of C-vitamine, which is in direct opposition to the needs of most mammals. Regarding the requirements of grown pigeons for vitamine A, this must be left for the future to determine. Should it appear that adult pigeons also need vitamine A, it would be worth[6] while to so change the usual diet used to demonstrate the onset of

[5] Among these were also pigeons which after being cured of beriberi, still showed normal fecundity.

[6] Experiments were made by us (273), in which two pigeons lived for 49 to 54 days respectively on an artificial diet if B-vitamine was injected every few days, and finally died of sepsis caused by contamination of the vitamine solution. Later on, Stepp (274) carried out some similar experiments in which he kept a pigeon in excellent health for 91 days on dog biscuits extracted with alcohol, and rice polishings (orypan); when the orypan was discontinued, the bird died in 37 days. In spite of the use of orypan, the bird could not fly well and lost some weight.

beriberi, namely, white rice, that it would lack nothing but B-vitamine, although we were able to induce a typical beriberi with a synthetic diet lacking vitamine A (275). As we have already pointed out in the case of the chicken, all these dietary deficiencies can complicate the picture of experimental beriberi unnecessarily. Because of the

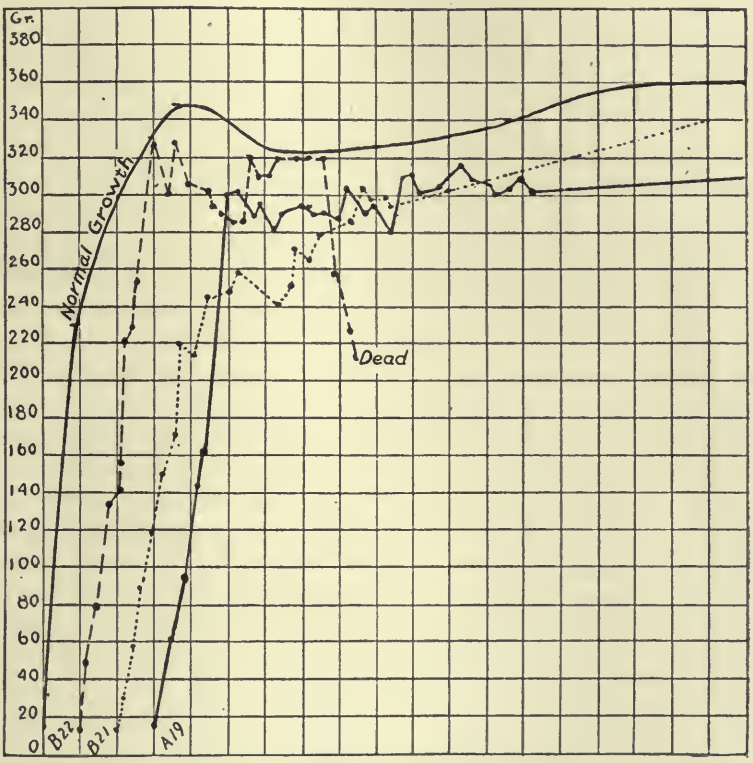


FIG. 12. GROWTH CURVES OF SQUABS

Parents were fed on a diet limited to the corn and wheat kernel, supplemented by the addition of calcium carbonate (oyster shells) (Voegtlin-Myers).

protein deficiency of white rice, Dutcher (276), and others, used an addition of 3 per cent casein, calculated on the dry weight of rice.

Pigeon beriberi. The method most commonly used to induce beriberi in pigeons is to feed white rice, although other foodstuffs and mixtures of isolated substances are available for that purpose.

Among others, Funk and Cooper (277) showed in 1911 that the disease could be brought about with pure sugar, inulin, dextrin and starch. The pigeon is quite the best animal to use in the study of beriberi. When pigeons are placed upon a diet of polished rice, they eat it with great avidity during the first few days. Following this, their appetite diminishes markedly, and they frequently attempt to disgorge the rice out of their crops. This behavior is more often noticed in animals that are fed forcibly, but sometimes this rice is again eaten voluntarily. This condition makes it difficulty to carry out exact rice feeding experiments with pigeons. One has the impression that the animals have an aversion for rice, which, however, disappears if the necessary vitamine is administered at the same time with the food. After a few days, it is best to put the pigeon in a box with a wooden cover so that its head extends; in this way it is possible for one person to manage when forcible feeding must be resorted to. The beak is opened and through a small metallic funnel with smooth edges, the weighed rice is pushed into the crop with a glass rod. Another method used by us was to prepare the food in the form of pills, and feed them as such. For feeding purposes, it is best to use healthy males weighing from 300 to 350 grams; 20 to 30 grams. of rice per day may be given in three portions. If the crop should happen to be full, it is necessary to wait till it is empty. We shall speak of the effect of the food ingested when we come to the physiology of vitamine B (p. 210). After several days of rice feeding, a marked change is noted in the animals. ' Very little remains of the usually predominating desire to quarrel when a number of pigeons are kept in one cage. Likewise, the sexual instinct, such as the strutting of the male around the female, seems to be held in abeyance, and the animals sit on the perch in a sleepy, apathetic manner. They experience increasing difficulty in flying on to the perch and a few days later some of the pigeons sit on the bottom of the cage, regardless of the fact that they are soiled by the feces of the animals on the perch above. The fecal matter, normally of semi-solid consistency and whitish color, becomes slimy and water-clear, or slight yellowish, and the animals show no disposition to keep themselves clean. If they are left to feed themselves, their appetite disappears gradually and their condition is that of semi-starvation. About 30 per cent of the animals which are permitted to feed themselves develop symptoms of beriberi, while the remainder die of general weakness. Of

the animals forcibly fed, a greater proportion develop the typical beriberi symptoms, after a period of time which is subject to great deviations. We may say, however, that most of the animals develop the disease in from 10 to 30 days; it is noticeable that the animals mostly become sick within a few days of each other, so that there is the false impression of the appearance of an epidemic. In the individual animals, we note the development of various types of diseases, as illustrated in the accompanying pictures, closely resembling the conditions described in man. This analogy may well be fallacious and the types described below may depend perhaps only on the nutritive condition and the length of time the rice is fed.

In one type, which we shall designate as acute, we notice that the ability to walk is decreased. If the animal is frightened, it runs a few steps without difficulty, but when fatigue sets in, the animal helps itself by flapping its wings, but sits motionless when left alone; the heart beat is pronounced and behaves as though it had been overexerted. The next symptom is uncontrolled motions of the head. If the animal is turned about several times in the air, then in many cases the acute form is produced out of the latent. The phenomenon appears quite suddenly—the head is pressed against the back by a retraction of the neck muscle, the legs are drawn up to the belly; the animal turns somersaults, and this may last a long time under certain conditions. In this stage, the animal does not survive very long. During the course of a day, a disturbance in breathing develops; the animal opens its beak wide, apparently struggling against suffocation, and finally death results in from 12 to 24 hours after the appearance of the symptoms, with an easing up of the spasms.

The second or chronic type (figs. 14-15) develops as follows: After a few weeks the animal is found sitting in the cage; it moves very reluctantly in spite of its ability to do so. After some days, it loses even this capability, and sits motionless in one spot. Only seldom does this form go over into the spastic. Usually, the animal lives a few weeks longer and dies without moving from its place. These cases are not suitable for curative experiments. For this purpose, we must use only those animals in the first group.

The latter also show variations. In some, the spastic condition persists till death while in others, it disappears for short intervals only to reappear in a more severe form.

FIG. 13. SPASTIC FORM OF BERIBERI IN PIGEONS

FIG. 14. ATROPHIC FORM

FIG. 15. PARALYTIC FORM

FIG. 16. SPASTIC-PARALYTIC FORM

A further phenomenon, common to both forms of beriberi in pigeons, is the loss in weight, which is apparent before the onset of the symptoms. This loss, amounting to from 20 to 45 per cent of the original weight, occurs always in pigeons fed on rice, according to our experience. The loss in weight is apparent whether the animal is fed forcibly or not. Results to the contrary are due to the fact that the pigeons, chiefly because of the paralysis of the muscles of the crop, are unable to empty it. On post mortem, the crop is tightly packed with rice, weighing about 100 grams, which ordinarily would have been calculated as body weight. It cannot be emphasized sufficiently that in experimental beriberi, following a strictly managed

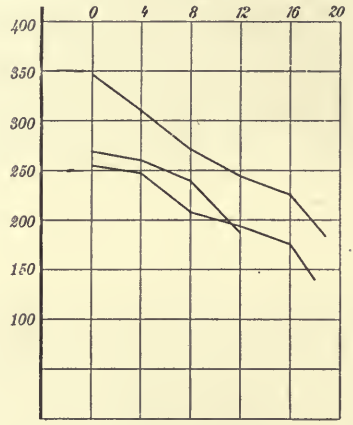


Fig. 17. Drop in Weight of Pigeons Fed on 30 grams Polished Rice Daily

diet, death ensues in 100 per cent of the cases. Theiler, Green and Viljoen (278) have observed that starving pigeons, only on water, may show spontaneous cures. This behavior was explained by the mobilization of the vitamine in the tissues. The tissues are catabolized more rapidly during starvation and therefore a greater supply of vitamine may suddenly be made available for the animal. If rice feeding is continued death must eventually ensue. The question of malnutrition in beriberi was also taken up by Lumière (279).

Besides chickens and pigeons, beriberi has also been described in other birds, which might sometimes also be of practical importance. For instance, Merklen (280) described a disease in 3 or 4 weeks old ducks manifesting itself in symptoms of cramps, paralysis of the legs

and inanition, which quickly disappeared on substituting the diet by one more varied. Perhaps we are dealing with beriberi here too. The appearance of beriberi in other birds is tabulated below:

Animal	*Observer*
Ducks	Eijkman (l.c.48) and Külz (281)
Geese	Eijkman (l.c.48)
Sparrows	Fujitani (282)
Quail	Toyama (283)
Jushimatsu	Toyama (283)
Parrots	Fink (284)
Rice birds	Ottow (518)
Munia maja	Jansen (284a)

Pathological anatomy and chemical pathology of beriberi in birds

Pathological anatomy. Till recently, everyone was of the opinion that in avian beriberi the manifestations in the central nervous system were in the foreground. The new investigations show, on the other hand, that as a result of the lack of vitamine B, many, if not all, of the organs suffer, and for this reason the disease cannot be thought of as "polyneuritis." Despite many attempts, it has not yet been possible to find out which organ or tissue is primarily responsible for the manifestations of the disease. This is of course bound up with our insufficient knowledge of the physiology and significance of the vitamines.

Nervous system. We find a very good description of it in the work of Vedder and Clark (l.c. 266), already mentioned, and more recently in a report by Onari Kimura (285). We see from these investigations, that not all nerves undergo pathological changes in the same measure; for example, the vagus degenerates, but not in a high degree. However, in the opinion of Kimura, beriberi is a general disease of the nervous system, and this was our opinion too. We still believe that all of the heterogeneous pathological changes occurring in beriberi may best be looked upon as of central origin, in so far as they may all be regarded as atrophic changes. Naturally, we admit that still another interpretation may be found.

The nerves of the lower extremities are selectively more affected than those of the upper. Especially is this true of the sciatic and peroneus nerves which show a fatty degeneration, though no definite

paralysis is noted. These changes arise after 7 days of rice feeding. The number of degenerated fibers bears no relation to the severity of the paralysis. Cases of light clinical symptoms often display marked degeneration, while severe cases frequently show only from 4 to 10 per cent of degenerated fibers. As a rule, from 10 to 15 per cent of the total fibers are found changed. Schnyder (286) on the contrary, finds only slight degeneration in birds and believes, because of the therapeutic influence of the vitamine, that the paresis could not be the result of degenerative processes in the brain. We have frequently pointed out that if we kill an animal a few days after it has been cured with vitamine, and make a histological study of the nerves, the degenerated fibers are still to be seen and persist for a long time. In the meantime, the normal fibers appear to assume the functions of the diseased fibers.

The nerves, histologically examined, give the following picture: According to Kimura, the first indication of degeneration is to be sought for in the axis cylinder, while the earlier prevalent idea was that the signs of degeneration are first noted in the medullary sheath; the myelin sheath, according to Kimura, may remain intact. However, as soon as the latter degenerates, the axis cylinder can no longer be differentiated. The myelin fragments thereupon are resorbed in situ through the cells of Schwann's sheath. If a degenerated myelin fiber still contains an axis cylinder, it is a regenerated axis cylinder from the ribbon-like protoplasm. The new cylinder is smooth and fragile and resembles that seen after a trauma.

The same changes are also to be noted in the dorsal and ventral nerve roots, as in all the dorsal strands of the spinal cord. Further, changes are apparent in the cells of the forward and rear horns of the lumbo-sacral cord; here the tigroid bodies are invisible, and the stainable substance (Nissl's method) accumulates over the axis cylinder. In some cases, the nuclei are weakly colored. Similar pathological nerve studies were made by Weill and Mouriquand (287), Kato and Shizume (288) and Paguchi (289).

The muscles. The muscles exhibit atrophy and fatty degeneration, but the changes disappear rapidly on returning to normal nutrition.

The heart. In most cases, this is unchanged; only seldom is there any edema, pigmentation, and traces of parenchymatous degeneration. Hypertrophy of the right heart, as in human beriberi, does not take place in chickens. On the contrary, we have personally seen

hydropericardium in pigeons. McCarrison (290) attributes the edema to the enlargement of the suprarenals.

Endocrine glands. The first investigations in this direction were made by Funk and Douglas (291). The findings in pigeons showed that the thymus glands disappear; this was also noted by R. R. Williams and Crowell (292). The behaviour of this gland in birds was then investigated further by McCarrison (293). Funk and Douglas made a histological investigation of pituitary, adrenals,

FIG. 18. FATTY DEGENERATION OF SCIATIC NERVE IN CHICKEN BERIBERI

ovary, testicle, kidney, liver, pancreas and spleen. In all these organs, definite signs of degeneration were noted. The investigation of thyroid in greater detail was made by Douglas (294) who observed carefully the great variations and the tendency of the colloids to disappear from the vesicles. McCarrison (295) studied the influence on the thyroid of food poor in vitamine, and found that the size of the gland, as well as the weight, decreased. The study of the endocrine glands of pigeons was then undertaken by the same worker (296) who largely corroborated the work of Funk and Douglas, and found

that the organs undergo atrophy in the following order: thymus, testicle, spleen, ovary, pancreas, heart, liver, kidney, stomach, thyroid and brain. McCarrison made the very interesting and important observation that the suprarenals are often hypertrophied,[7] and that this hypertrophy bore a causative relationship to the onset of edema (l.c. 290). He believed that in unpolished rice, butter (particularly in butter, as will be noted later) and onions, there was a substance which was protective against edema, and which might be thought of as vitamine A.

McCarrison found also that the brain, suprarenals and the pituitary, are very sensitive to a lack of vitamine. The sexual organs in males showed a disappearance of spermatogenesis, with resulting sterility; in females, there was a condition similar to amenorrhea. This is in opposition to the fact that we mated pigeons, cured of beriberi, and secured a completely normal progeny. It is nevertheless possible that in these cases, there may have been a regeneration.

During the course of his fruitful investigations, McCarrison also examined the muscles and found them highly atrophied, while the central nervous system was only slightly so; he attributed the resultant paralytic symptoms directly to the impaired functional ability of the nerve cells. Because of the remarkable atrophy of the thymus, testicles, ovaries, and spleen, much more apparent than in other organs, resulting from the lack of vitamines, he believed that these organs provided the distressed organism with the needed vitamine. When this reserve is exhausted then the bones are the purveyors of the vitamine, in which case the marrow undergoes marked changes. Red corpuscles are also diminished about 25 per cent. Finally, McCarrison regarded the whole picture of beriberi as a syndrome, arising from, (1) a chronic inanition; (2) a pathological change of the organs of digestion and assimilation; (3) an abnormal functioning of the internal secretory glands, especially of the suprarenals; and (4) poor nutrition of the central nervous system. In addition to all these factors, there is also a decreased resistance to the bacterial invasion. McCarrison (297) at first erroneously attributed to the latter condition the entire picture of beriberi, but he has long since abandoned this view. The pathological histology of beriberi in pigeons is described by McCarrison (298) in a special article.

[7] Unfortunately, in the pathological department, which took up this phase of the work, this observation completely escaped notice.

Changes in the gastro-intestinal tract. McCarrison's (299) investigation on 153 pigeons yielded some very interesting results. The upper part of the intestine, which is muscular in nature in the region of the pancreas, is mostly involved. Atrophy had proceeded to such an extent that the walls were almost transparent, especially when white rice was fed for a long time. The intestine showed a

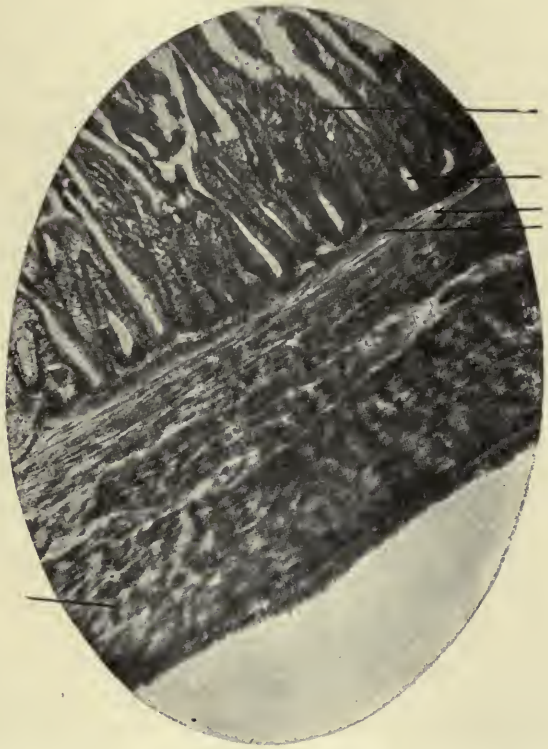


FIG. 19. THE UPPER PART OF THE INTESTINE OF A HEALTHY PIGEON (McCARRISON)

definite congestion; ecchymosis was not infrequently seen and microscopic hemorrhages were almost always observed. Under the microscope, the congestion led to a partial disappearance of the epithelium. The myenteron was so severely atrophied, that its motor functions suffered. Further, there was described degeneration of the mesenteric plexus of Auerbach, which impaired the functions.

of the intestinal nerves. The villi disappeared and there was atrophy and inflammation of the mucosa and atrophy of the lymphatic tissue. The latter condition is primarily responsible for the decreased resistance to infection. According to McCarrison (300), the severe intestinal infection is the chief reason why some animals cannot be

FIG. 20. SECTION OF UPPER PART OF INTESTINE FROM A PIGEON DYING OF BERIBERI

Thinning of muscular coat, intense atrophy and necrosis of the mucous membrane.

saved by an addition of vitamine. Pigeons fed on autoclaved rice, butter and onions develop the same pathological picture, with the difference that the changes are not so far advanced. After the elimination of butter, it may be shown that onions have the power to protect the intestinal canal against degeneration. By the addition of peas, containing vitamine, the pigeons may be cured,

while the pathological picture of the intestine remains the same. In his opinion, the cure is so rapid that it cannot be due to the vitamine absorption; the therapeutic effect may be compared, in its velocity, only to the sudden manifestation of the growth impulse in germinating seeds.

Chemical pathology. The chemical investigations are not yet so numerous. Schaumann (l.c. 2) investigated the brains of a beriberi-pigeon and found that the phosphorus content was not decreased. In contrast to this, we (301) found that the nitrogen and phosphorus content of these brains is below the normal content; indeed in normal pigeons, we found 9.77 per cent nitrogen and 1.84 per cent phosphorus, while in beriberi pigeons, there was 9.31 per cent nitrogen and 1.53 per cent phosphorus. Our results were confirmed by Wieland (302) and by Mathilde Koch and Voegtlin (303), although it is not certain just how ordinary starvation would affect these figures.

Funk and v. Schönborn (304) also showed that in pigeon-beriberi, there is an increase in blood sugar and a decrease in the glycogen content of the liver almost to the point of complete disappearance. Because of this, we were very much inclined to bring these facts into causative relationship with the observation of McCarrison on hypertrophy of the adrenals.

It must also be recorded that Fuji (305) found the diastase content of the blood decreased in chickens suffering from beriberi; on administration of vitamine, this value returned to normal. Analogous results were obtained with glyoxalase of the liver by Findlay (305a).

MAMMALS

In 1913, we stated that certain animals do not develop beriberi when they are put on a diet of white rice; we attempted to explain this by its dependence on the specific purine metabolism of the particular species. With this in view, we classified mammals into two broad divisions, one in which the end product of purine metabolism is uric acid, and the other in which it is allantoine. The first division included man and birds, manifesting real beriberi, while the second included the rat, the dog,[8] the monkey—which can contract

[8] Bearing upon this, it would be interesting to determine if a different reaction to the lack of B-vitamine is manifested by Dalmatian dogs, which, according to S. R. Benedict (307), excrete uric acid chiefly instead of allantoine, and by anthropoid apes, found by Hunter and Ward (308) to have a purine metabolism similar to that of man.

scurvy—while guinea pigs with their characteristic purine metabolism occupy a middle position. Whether these conceptions may still be regarded as correct is not certain, for in the first place, a type of beriberi in dogs and rats has been described and secondly, there is a possibility of another explanation of the above mentioned observations. At all events, we wish to leave the question open for further investigation, particularly since beriberi has not been definitely demonstrated in certain animals, and may even be confused with other pathological conditions. The criteria for the recognition of beriberi, such as the fatty degeneration of the nerves, are not specific symptoms of beriberi. We have fed white rice for a long time to rats, rabbits and guinea pigs, without noticing any kind of a symptom to suggest beriberi. The other explanation, of the different behavior of certain animals on a diet of white rice, we find in the unknown rôle of the bacterial intestinal flora, the significance of which has already been mentioned (p. 76) in the chapter, "Life Without Bacteria." We come now to the consideration of the importance of the vitamines for various mammals.

Rats

Rats have been extensively used as experimental animals in the last 10 years in the attempt to solve the problems of nutrition, and for vitamine research. The reason for this is that rats breed easily, do not consume much food and are not particular in their taste. The fact that a greater number of these animals may be used at one time for an investigation makes the results much more certain. Since in most problems of nutrition, the food must be specially prepared, as a rule, the small amount ingested is of practical significance. Rats live about 3 years and reach sexual maturity very early. The pregnancy lasts 3 weeks and the offspring is numerous; the number of young varies from 6 to 12 according to the age of the parents and their nutritive condition. The young rats begin to eat of their own accord after 3 to 4 weeks, and may be used for feeding experiments when they weigh 30 grams, though it is better to wait till they weigh from 40 to 60 grams. It has been shown by many investigators, among them McCollum, Simmonds and Pitz (309), that inadequacies of the diet are manifested in a diminished milk secretion of the mother, or else in a poor state of health of the young, although the parents show no such signs. It is therefore desirable, if one

wishes to demonstrate the nutritive value of a certain diet to carry out feeding experiments with the same material on the next generation. For such a purpose, the rat is naturally specially suited. Thus, it is possible to study, in a short time, not only the effect of the diet upon the parents, but also to extend the observations on the procreative power, the number of living young, and the lactation of the mother. Judging by our experience, we deem it inadvisable to

FIG. 21. RAT CAGE (MACALLUM)

6, water cup; *7*, flask for urine collection; *8*, bulb by means of which urine, is separated from feces (urine flows on the outside of the bulb into the flask and feces falls into beaker); *9*, food cups of various sizes; *10*, funnels of various sizes adapted to the age of the animal, so that the food may not be scattered.

buy rats for experimental purposes; it is far better to raise them in the laboratory. In this way we have the advantage of knowing the parentage of the animals to be used. When one obtains a litter, it is sometimes difficult to differentiate between the male and female. To obviate this, we can utilize a method described by Jackson (310). It is likewise of importance to use a cage so arranged as to be able to control accurately the amount of food given, especially since rats

have a tendency to scatter their food. This difficulty may be overcome if we use such a cage as has been described explicitly by our former co-worker, A. B. Macallum (311). The cage is equipped with funnels of various sizes through which the rat may reach its food without scattering it. The cage is arranged for two rats and permits of separating the urine from the feces, so that an exact metabolism experiment may be carried out.[9] Great care is necessary in the preparation of the diet; above all, it is important that the basal diet is vitamine-free. This precaution was not sufficiently observed in the older investigations, thus giving rise to differences of opinion. We need only quote one example, lactose, widely used as a constituent of an artificial diet. Regarding this, we showed[10] that lactose, which does not undergo any particular purification process, contains traces of nitrogen. These traces may come from the milk and permit of the growth of rats, because of its vitamine content. This was demonstrated experimentally by McCollum and Davis (l.c. 97) and also by Drummond (313). This is likewise true of the protein of protein-free milk, as we have already seen. It is necessary to subject the protein to be used to a purification process before it is fed. In most feeding experiments casein is used, and it is rendered vitamine-free by extracting with alcohol a number of times, according to the procedure of Funk and Macallum (l.c. 81); we found that this procedure did not decrease the nutritve value of casein, in spite of statements by McCollum and Davis (l.c. 97) to the contrary. It is, of course, possible to purify the casein by dissolving it in alkali and precipitating with acid, and also by continued washing with water. In using meat, according to Osborne, Wakeman and Ferry (l.c. 102), it suffices to extract it repeatedly with water. From edestin, as we have seen, this procedure removes the vitamine only with great difficulty. Of the other commonly used dietary constituents, cane sugar is vitamine-free, as are also most of the varieties of starch. Regarding fat, we have used ordinary lard and under certain conditions it can be entirely dispensed with. The purification of lard is made in our laboratory by autoclaving at 30 pounds for 3 hours; we shall have occasion to show later that this lard is then vitamine-free. For the entire dietary mixture, about 3 per cent of agar is added to diminish the possibility of constipation. If it is desired to use a sub-

[9] Ferry (312) describes the technique of rat feeding experiments.

[10] The Vitamines, 1st ed., p. 159.

stance hitherto not utilized, it is necessary, by means of a specific control, to test for the presence of vitamines. For an inorganic salt mixture, that described by Osborne and Mendel, somewhat like the inorganic salts of milk, or the one used by McCollum and Simmonds is available. The mixtures have the following composition:

Osborne and Mendel (314)		*McCollum and Simmonds (315)*	
$CaCO_3$	134.8	NaCl	0.173
Na_2CO_3	34.2	$NaH_2PO_4.H_2O$	0.347
H_3PO_4	103.2	K_2HPO_4	0.954
H_2SO_4	9.2	$CaH_4(PO_4)_2.H_2O$	0.540
Citric acid · H_2O	111.1	Iron citrate	0.118
$MgCO_3$	24.2	Calcium lactate	1.390–1.300
K_2CO_3	141.3	$MgSO_4$ (anhydrous)	0.266
HCl	53.4		
Iron citrate · $1.5H_2O$	6.34		
KI	0.02		
NaF	0.248		
$K_2Al_2(SO_4)_2$	0.0245		

The food mixture is best prepared so that it has more or less of a pasty consistency, in which form it is least scattered by the rats. As an example of a diet now thought to be complete, one having the following composition is given:

Substance	*Per cent*	*Substance*	*Per cent*
Casein	22	Lard	20
Cane sugar	10	Agar	3
Starch	27	Salt mixture	3
Butter	10	Yeast extract	6

In older investigations, for example, in the work of Hopkins in 1912 (l.c. 70), or even in that of Funk and Macallum (l.c. 86), not only was the amount of food consumed taken note of, but it was estimated how much of the food intake was assimilated, by means of calorimetric determinations of the food and of the excreta. In the newer investigations, the tendency has been to get away from the above time-consuming procedure, and to dispense with records of the quantity of food ingested. The rat has become, so to speak, a reagent for the testing of the value of the diet, and since many thousands of experiments have been made in the last 10 years, the investigators sought to simplify the experimental conditions as much as possible. Nevertheless, the weighing of the food seems to us to

be of the greatest significance, particularly in those experiments yielding negative results. It does not suffice merely to feed the animal; one must also be certain that it eats enough. If no food control is provided for, it is easy to make false conclusions; it would be possible to designate a certain diet as insufficient, whereas the true reason for the misleading experiments lies in the unsuitability of the animal. This applies in general to rats that are bought; it may be worth while mentioning that we have had particularly good results with black and white rats, raised in the laboratory; this strain seems to be unusually resistant.

It must be emphasized once more that not all problems of nutrition can be solved by the use of rats as experimental animals. To illustrate, Osborne and Mendel (316) have shown that when the growth of rats is prevented by a specially chosen diet, growth may be resumed after they have reached two-thirds of their size, upon the addition of the missing factor. While Jackson and Stewart (317), in confirmation of these findings, concluded that this ability to resume growth, after a period of repression, depends upon the age of the animals and the duration of the growth inhibition (in agreement with the work of Brüning (318) and Aron (319)), and under certain circumstances may be appreciably affected, we must nevertheless admit that the rat does possess this ability for the most part. Now, it would be futile to try to achieve the same results in man or other mammals of a corresponding age. As a matter of fact, various kinds of animals behave quite differently. In the same way, it is evidently impossible to study the nature of scurvy and pellagra in these animals as some investigators have done. The individual animals do not compare in regard to nutrition and nutritional diseases.

An interesting question is involved in experiments with inadequate diets to show how far rats have the power to choose out of two diets, the one that is adequate. This was investigated by Slonacker (320) with a primitive method, and repeated later by Osborne and Mendel (321). In the latter experiments, it could be shown that the rats chose correctly almost invariably.

The vitamine requirements of rats. Although the individual phases of this question have been briefly considered in the historical part and other sections of the book, it appears necessary to treat systematically this matter which has assumed such practical as well as theoretical importance. We have already said (even though the question should

not belong to the subject of vitamines) that for the correct feeding of rats, it is necessary, above all, that the food should have the proper composition. We know that a protein is necessary whose content of amino acids is above reproach. We may say that casein, lactalbumin,[11] egg-albumin, muscle protein, as well as some plant proteins have been shown to be adequate for this type of animal. Suzuki and his co-workers (323) demonstrated that an artificial mixture of amino acids does not permit of growth in the rat, while digested meat (erepton) possesses this ability because of its vitamine content. Aside from this, it may be connected with a more favorable relationship of the amino acids to each other. In addition, we refer to the excellent work of Osborne and Mendel, who have carefully studied the value of various proteins on rats.

Furthermore, it is necessary that the rats receive a sufficient and properly composed salt mixture, and that the individual constituents of the diet are present in proper proportion. This point, to be mentioned again shortly, is of great significance. It cannot be said with certainty that these basic conditions are always accurately maintained; it is only in the work of the last six years that they have been given their true value. All of the above mentioned factors must be taken into account before undertaking a vitamine problem.

We have already called attention to the work of Osborne and Mendel in 1911–1912, in which they still clung to the idea that they had solved the question of artificial nutrition. At first they did not see that the addition of "protein-free milk" to the diet was an unknown factor whose significance for nutrition could not be accurately gauged at the time. This happened in spite of the work of Stepp, who had already affirmed the presence of essential lipoids in milk. Through the classical work of Hopkins and through our (324) simultaneous demonstration of vitamine B in milk, it became clear that something could adhere to the products made from milk; this might explain the striking results obtained by Osborne and Mendel. Hopkins showed that a small quantity of milk (a few cc.),

[11] Sure (322) has recently found that lactalbumin is not a complete protein since it does not contain enough cystine and tyrosine. According to this, it is obvious that the results of Emmett and Luros (l.c.105), who suggested the probability of a new vitamine, and who found that lactalbumin was rendered complete when supplemented with protein-free-milk, could be explained by the presence of cystine and tyrosine in the protein-free milk.

added to a carefully purified food mixture, resulted in normal growth of rats; at the same time the food intake increased markedly. The favorable effect of these small amounts of milk is best shown in the accompanying curve. Since Hopkins fed milk in the natural state, its effect could be due to the cumulative influence of several unknown factors—which was found later to be the case. Subsequently, Funk (325), and also Osborne and Mendel (326), showed that milk does

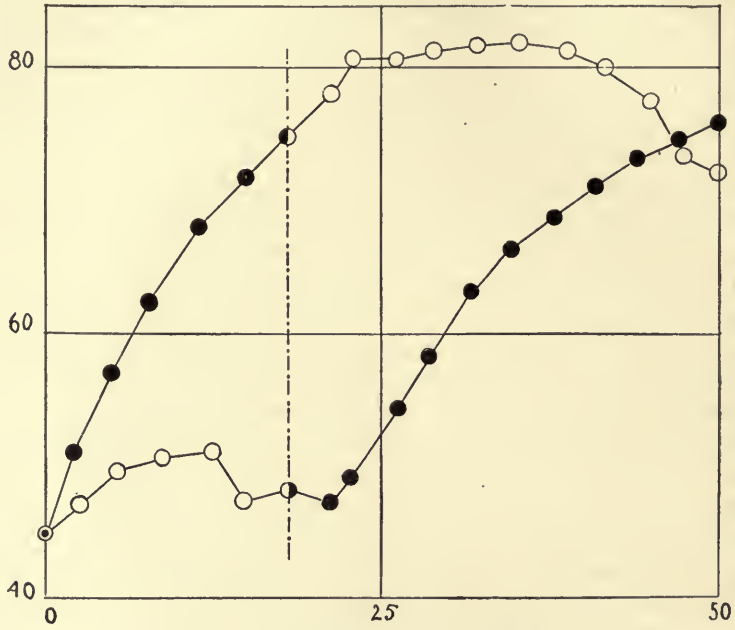


FIG. 22. GROWTH CURVES (HOPKINS)

Lower curve (up to eighteenth day), vitamine-free diet; then 3 cc. milk daily; upper curve, the reverse.

not exert such a great influence as Hopkins first found; however, we must not forget that the vitamine content of milk and other food stuffs does not represent a constant mathematical unit and can undergo great variations.

McCollum and Davis (l.c. 75), and shortly thereafter also Osborne and Mendel (l.c. 76), showed that protein-free milk was not sufficient for the growth of rats, and that the diet needed certain fatty constituents, found in egg-yolk and in butter. The lack of these factors

was evidenced by a general undernutrition, a rough coat and a characteristic eye disease, now called ophthalmia (keratomalacia). Since this disease does not occur only in rats, we shall have occasion to speak of it again. It was difficult to bring these new findings into accord with the then prevailing knowledge of vitamines, the more so since Osborne and Mendel (l.c. 72) obtained good growth with fat-free mixtures. Later on, Stepp (327) showed that his "life-sustaining" substances did not belong to the lipoids, and Lander (328) demonstrated that lecithin and cholesterol played no particular part in nutrition. Hence we could not understand why the rôle of vitamine B was relegated to the background, and even denied. Subsequently, Aron (329) also reported on the importance of butter.[12] Although we had already at that time assumed the existence of a special vitamine, associated with fats (in cod liver oil, we assumed the existence of a specific antirachitic vitamine because of the partial lack of vitamine B and the therapeutic influence on rickets) we were somewhat skeptical of the existence of a vitamine in butter. In this we were strengthened by our experiments together with Macallum, particularly since butter was shown to be free from B-vitamine and since it was possible (330), by substituting fresh yeast for dry yeast, adding orange juice, and treating the eyes with zinc sulfate and boric acid solution, to maintain animals in good condition for 150 days. The substitution of butter for lard was without influence in our experiments. All these results are now easily explainable, and yet we must assert that we have observed instances of ophthalmia on a diet containing butter.[13] Our findings showed that the substance present in butter was not the only growth factor, as McCollum would have it, but that vitamine B is at least quite as important as vitamine A. This is well illustrated in the curve taken from the work of Funk and Macallum.

[12] Aron (l.c.319) introduced the method of giving the vitamines as a medicine, so to speak, independent of the diet. This procedure has the advantage that the vitamine addition has nothing to do with the food consumption, although it is not always easy to give the necessary dose to the animals, as we ourselves can verify.

[13] Lately, in association with Dubin (331), we have noted, among 30 rats, 2 that developed ophthalmia on a diet containing the usual amount of vitamine A. One of them was improved by the addition of yeast to the diet. The other improved without medication.

Effect on rats of the lack of vitamine B. If a diet is chosen, complete in everything else but lacking in vitamines A and B, a nitrogen balance can be maintained for 24 days, according to the findings of Desgrez and Bierry (332). If the experiments are extended for a longer time, we obtain the following curve, reproduced in the Report

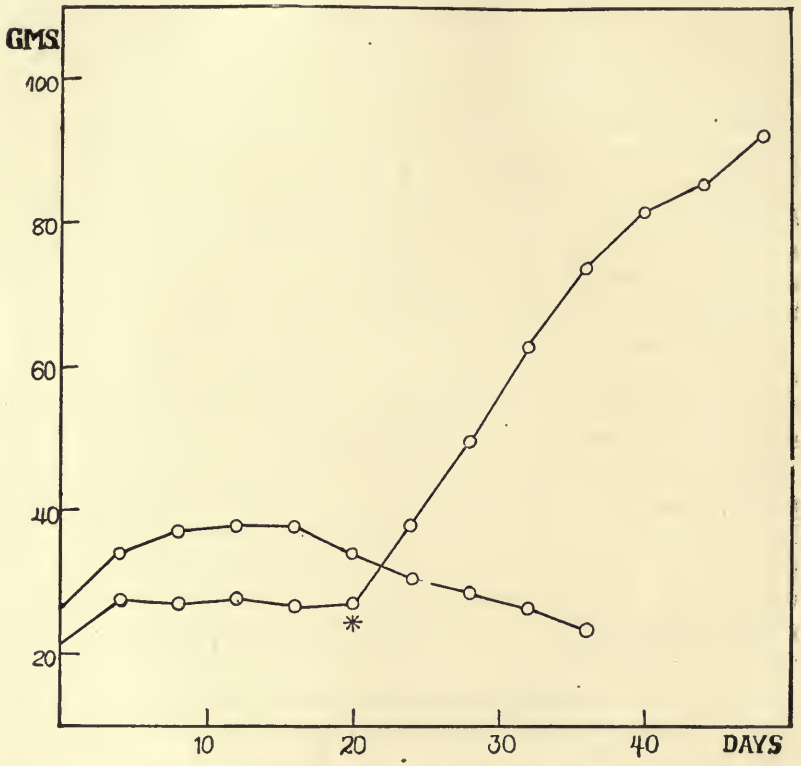


FIG. 23. SIGNIFICANCE OF VITAMINE B FOR GROWTH OF RATS

Vitamine A was present as butter; addition of yeast indicated by * (Funk-Macallum).

of the English Medical Research Committee (333) on vitamines. It may be seen that growth ceases completely if both vitamines are lacking, and the animals would have died if these factors had not been administered in time. The addition of vitamine A only had no influence on the growth, while the addition of vitamine B resulted in slight growth, which, however, stopped again after a short time.

The difference in the mode of action of both vitamines was explained by the supposition that there is only a small reserve supply of the antiberiberi vitamine in the body, and hence a lack of it is almost immediately noticeable; on the other hand, the reserve of antirachitic vitamine was thought to be greater. This observation may naturally be explained in another way, namely, that vitamine B is of greater significance in metabolic processes and growth, and hence larger amounts are necessary. In accordance with this, is the fact that grown rats require less vitamine A than young rats, whereas vitamine B is necessary throughout the course of life. When there is a lack of vitamine B, symptoms arise which have been likened to those of beriberi by Funk and Macallum. Be that as it may, these symptoms

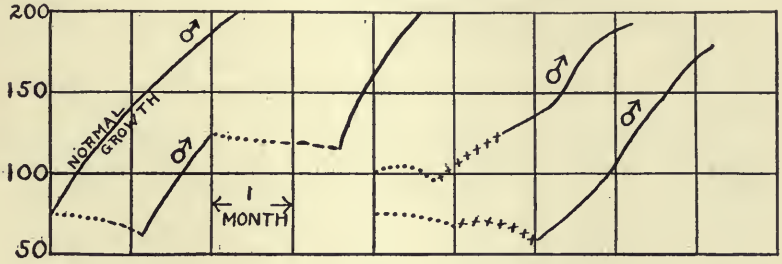


Fig. 24. Growth of Rats Showing the Dependence on Vitamines A and B. (Rep. Med. Res. Com.)

..... Diet without vitamine B
——— Complete diet
– – – – Diet without vitamines A and B
x x x x Diet without vitamine B

may be obviated just as in avian beriberi. Whether it really is beriberi remains to be demonstrated. A paresis of the legs is no rare occurrence in these rats. Voegtlin and Lake (334) observed that rats are much more resistant to beriberi than are cats and dogs. Since beriberi is not an infectious disease, we cannot speak of a resistance; rather must we say that the lack of vitamine B in rats is not followed by characteristic symptoms. It is interesting to follow the influence of an addition of vitamine B upon the food intake, as is shown in an experiment by Osborne and Mendel (335). Osborne is of the opinion that a daily addition of 0.2 gram yeast is quite sufficient to provide for the vitamine B requirement of rats. Osborne and Mendel (336) rightly maintain that the effect of vitamine B

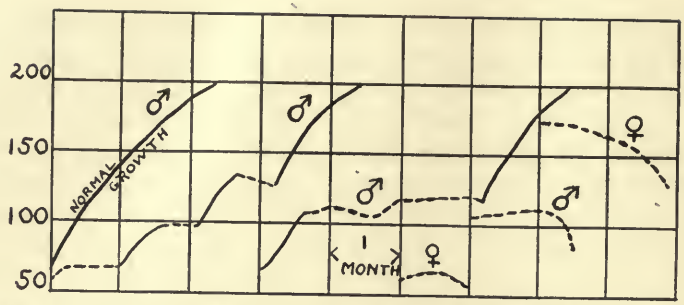


FIG. 25. SHOWING FAILURE TO GROW ON A DIET DEFICIENT IN VITAMINE B, AND RECOVERY ON ADDING THAT FACTOR (REP. MED. RES. COM.)

..... Diet without vitamine B
——— Complete diet

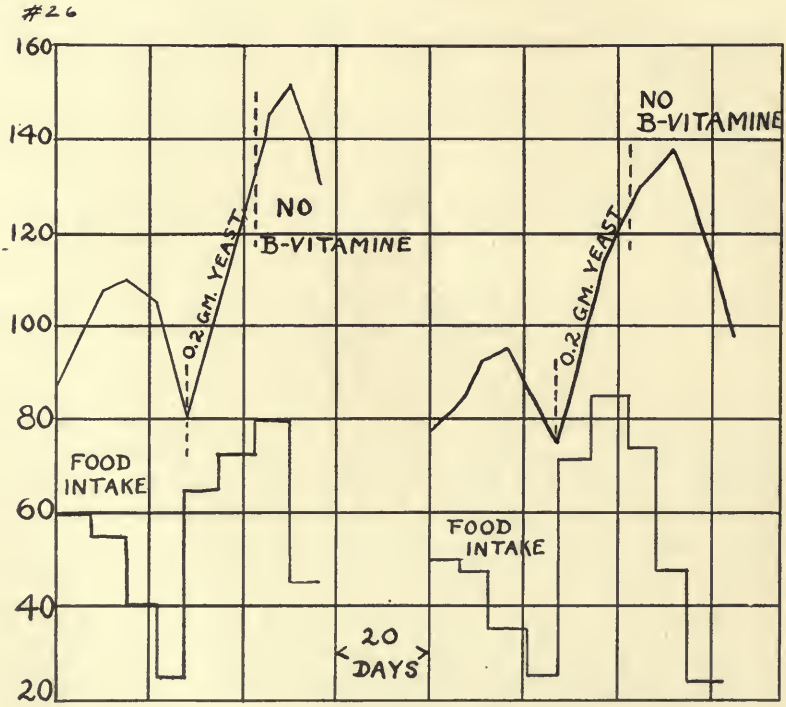


FIG. 26. INFLUENCE OF VITAMINE B ON THE FOOD-INTAKE (OSBORNE)

extends to the entire metabolism, and that the specific influence does not depend upon the food intake.

The effect of the lack of vitamine B on rats was studied by Drummond (337). Among other observations, creatinuria was noted; this was connected with the cleavage of muscle tissue. Grown animals often exhibited subnormal temperatures, while paralytic symptoms were rarely noted. The right heart in some cases was enlarged and the testicles showed a suppression of spermatogenesis.[14] Emmett and Allen (339) investigated the organs and glands of rats kept on a diet poor in vitamine B. They noted atrophy of the thymus and hyper-

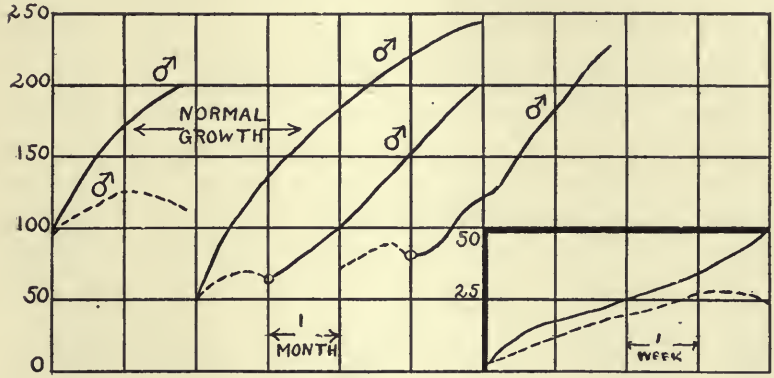


FIG. 27. SHOWING PRELIMINARY GROWTH AND EVENTUAL DECLINE ON DIET DEFICIENT IN VITAMINE A

Also recovery on addition of a source of vitamine A. In the small diagram, the lower curve shows growth of breast-fed young when the mother is fed on a diet deficient in A-vitamine; the upper curve shows the same when the mother is fed on a complete artificial diet. (Rep. Med. Res. Com.)

trophy of the adrenals, similar to the findings in birds. Liver, heart and other organs, particularly certain intestinal segments, were found inflamed and infiltrated. Frequently, a fatty degeneration of some organs was noted. Lack of vitamine A, however, led to no pathological changes, at any rate not of such a nature as were easily demonstrated. In this case, the symptoms were least apparent in older

[14] Osborne and Mendel (338) held that yeast as the sole source of proteins causes sterility in rats, but it still remains to corroborate this finding. These investigators confirmed our statement (l.c.325) that for rats, yeast protein can completely replace the protein of the diet.

animals. The animals showed a coarse and sparse coat, and a decreased resistance toward infections of all kinds, such as ophthalmia, and pulmonary affections, like pneumonia. Tsuli (339a) investigated the action of deficient diets on thyroid, gonads, parotid, pancreas and other organs. Aron (340) observed these conditions, including neuro-paralytic changes in the skin, to which he also attributed ophthalmia. Morgulis and Gies (340a) found the calcium content of rachitic bones and teeth lower than in the normal. Mattill (340b) compared the creatine, creatinine, and urea content of the blood in fasting rats and those deprived of B-vitamine. The non-protein N was increased in both cases—in fasting animals, mostly in the form of urea, while in the others, it was in the form of creatine. Brüning (340c) saw in rats fed on a carbohydrate-rich diet a pathological condition arise resembling "Mehlnärhrschaden."

A question that has greatly interested the author for a long time, was to determine if with both of the above mentioned vitamines, all of the nutritive requirements of the rat have been fulfilled. Looking through the many reports dealing with this problem, one is tempted to answer this question in the affirmative. First of all, we must see whether the growth that has been obtained till now on artificial diets, represents the optimum growth of rats. It is obvious from most of the growth curves that growth on the experimental diet compares favorably with that on a normal diet. To satisfy ourselves on this point, we (l.c. 325) followed the growth of a number of rats on a normal diet, which contained, among other things, yeast and condensed milk. The experiments showed that the growth, regarded by Osborne and Mendel and also McCollum as normal, was not the optimum for this type of animal. For comparison, we append the normal weight curves obtained by us, Osborne and Mendel, and McCollum. It should also be noted that in a large percentage of these investigations, particularly those of McCollum and his co-workers, still more complicated food complexes were fed, which, at least theoretically, might still contain one or more unknown factors. On the other hand, we must admit that in laboratories greatly experienced in rat nutrition, for example, those of Osborne and Mendel, the condition of the artificially fed rats, in relation to the state of health and the ability to rear their young, leaves nothing to be desired. The explanation here may be that through considerable experience a dietary composition has been chosen, the components

of which by chance may supplement each other. In our earlier work, we believed at first that we could conclude from the symptoms (disposition to bleeding, petechiae and rosary) and from the favorable influence of orange juice, that we were dealing with an antiscorbutic vitamine deficiency. Macallum (341) later adopted the same view. Recently, there appeared the papers of Harden and Zilva (l.c. 100) and Drummond (l.c. 79) completely corroborating the above observations. Still, some doubt has lately arisen, since Osborne and Mendel

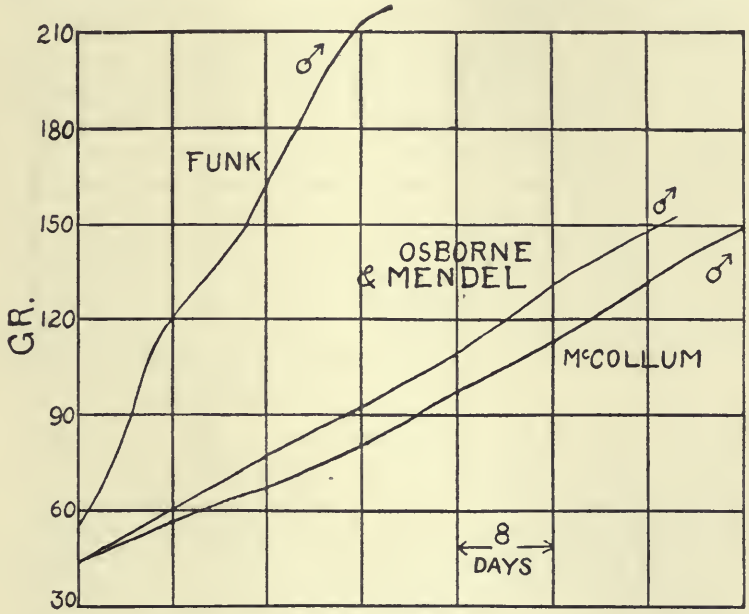


FIG. 28. COMPARISON OF NORMAL RAT GROWTH CURVES OF OSBORNE AND MENDEL, MCCOLLUM, AND FUNK

(l.c. 92) as well as Byfield, Daniels and Loughlin (l.c. 90), showed that vitamine B is found in appreciable quantities together with vitamine C in fruit juices. This is in agreement with our latest observations, noted in our work with Dubin, that rats on a diet containing sufficient vitamine nevertheless show improvement upon the addition of extra vitamine. Besides, in our earlier and later work we have often found that an addition of freshly prepared dietary mixture is followed by marked stimulation of growth.

Reviewing the many publications dealing with the growth of rats, it is evident that only strictly positive or strictly negative results are recorded. On the other hand, we have often seen rats that were not in the best of health after 90 to 100 days on a diet that could be regarded as complete in every respect. To give a concrete example.

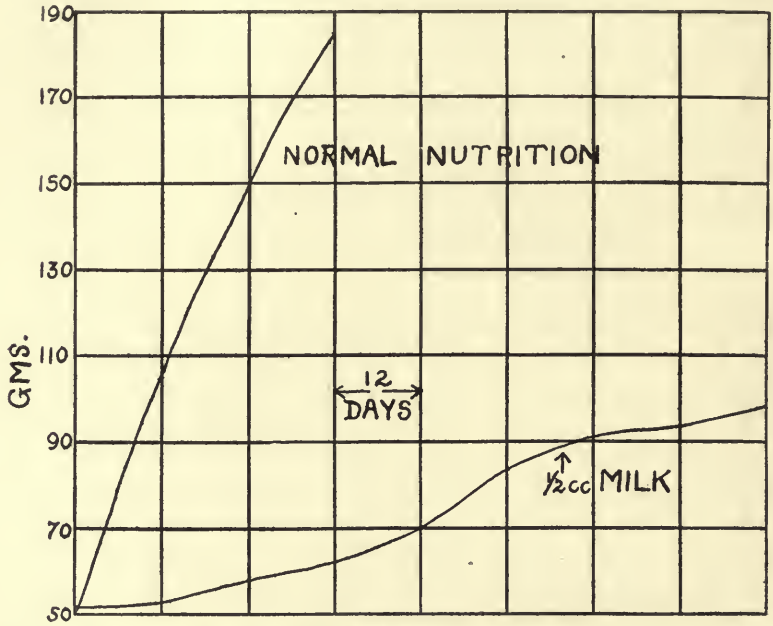


FIG. 29. RETARDED GROWTH SOMETIMES NOTED IN RATS ON A SUPPOSEDLY ADEQUATE DIET, COMPARED WITH THE NORMAL CURVE (FUNK–DUBIN).

we have only to record a diet used in the work of Funk and Dubin (l.c. 331):

Substance	*Per cent*	*Substance*	*Per cent*	*Substance*	*Per cent*
Extracted meat	22	Lard	20	Autolyzed yeast	4
Cane sugar	15	Starch	22	Orange juice	3
Butter	10	Agar	3	Salts	3

Despite the fact that this composition is above reproach, in the light of present-day knowledge of nutrition, our animals did not grow very well. Thereupon, we tried to bring about normal growth

by the addition of vitamines A and B, tomato juice, small quantities of milk, and by the substitution of casein for meat. Although some of these modifications were effective for a short time, the growth curve was nevertheless flat. That the animals were still able to grow, was demonstrated by placing them on the usual normal diet. Hopkins (341a) recently also reported that rats failed to grow on adequate food mixtures, and he ascribes this to the seasonal variation of the growth impulse. Osborne and Mendel (l.c. 336) also showed that it is not so easy to raise rats with an addition of yeast as with the addition of protein-free milk; it was particularly noticeable that there was a difference in the ability of the rats to raise their young. Sugiura and Benedict (l.c. 108) obtained similar results, as did also Freise (342), who ascribed a particular rôle to milk.

In collaboration with Dubin we (342a) have recently demonstrated that what has up to now been known as vitamine B does not appear to be sufficient for the growth of rats. Thus, it seems that autolyzed yeast contains besides vitamine B something else of a vitamine nature without which the rat does not grow. This will be discussed more in detail in the chapter on the "Chemistry of Vitamine B." Judging from the data recorded here, it appears desirable to study the nutritive requirements of rats still further.

Mice

As we have already seen, the pioneer work of Stepp, Röhmann, Lunin, Socin and others on artificial feeding was done with mice. Getting away from the older work, which has more of a historical interest, and coming to the modern investigations, we see in the work of Brailsford Robertson (343), statistical data in relation to growth of mice, which could be useful in judging the effect of experimental diets. Thompson and Mendel (344) studied the food requirements necessary for the growth and maintenance of the mouse, the suppression of growth, shown by undernourishment, and the amount of food necessary to overcome this suppression. Mendel and Judson (345) working with mice analyzed the total dry substance, fat and ash of various dietary constituents. M. B. Schmidt (346) made the interesting observation that if a deficient diet has no effect on the first generation in these animals, its results are apparent in subsequent generations. Morpurgo and Satta (347) worked with mice in parabiosis in which one animal received only cane sugar while the

other was given a complete diet. Both mice were kept alive, showing that the one animal received with blood, not only the usual dietary constituents, but also the vitamines.

Of the important investigations on mice, we shall first mention those of Stepp, upon whose work of 1909-1911 we have already touched lightly. He extracted the whole diet, thereby partly removing and partly destroying the vitamines. In this way, he obtained mixed avitaminoses that could be attributed to the lack of at least two vitamines. He proved (348) that lecithin, cholesterol, kephalin cerebron and phytin could not replace the substances extracted by alcohol-ether mixture. He found also that while the important substances could not be removed by means of the ether extraction alone; they could be removed with alcohol alone. When egg-yolk is extracted with alcohol, these substances go into solution, but not when acetone is used. Following this, Röhl (349) found that ordinary commercial lecithin contained these substances, while pure lecithin did not. In a publication appearing in 1914, Stepp (350) drew close to the prevailing view of the importance of two vitamines in the life of mice although he still thought of the fat vitamine as a lipoid. In 1916, Stepp (351) advanced a step further in that he used two different extracts, one of egg-yolk and the other of rice polishings (orypan). However, the experiments in this case were not very clear, since at first Stepp worked with a vitamine mixture (egg-yolk), believing he had to deal with a lipoid. Furthermore, he showed (352) that a mixture of lecithin, kephalin, cerebron, cholesterol and orypan, added to dog biscuit which had been extracted with alcohol, could at least maintain mice at a certain level. It is, however, not impossible that this was due to a cumulative action of vitamine A contamination. Dezani (353) conducted experiments for the purpose of determining the source of cholesterol. He fed mice on flour and casein, extracted with alcohol and ether. The animals died after 18 to 19 days, showing a 41 per cent loss in weight. Cholesterol was found in the feces, and Dezani (354) was of the opinion that mice could be kept alive on a lipoid-free diet. We may mention here one of the typical experiments of Röhmann (355), in which mice were raised on a diet of casein, chicken protein, nucleoprotein from liver, potato starch, margarine and a salt mixture. This food proved to be inadequate and the animals grew less than on milk and rolls. The substitution of vitellin for nucleoprotein did not yield any better

results, and Röhmann regarded this diet as adequate only for grown animals. Meat extract and dried meat powder had no particularly favorable effect; egg-yolk on the contrary, was favorable but not sufficient for growth, while egg-fat was not well tolerated, even in older animals. In a second series of experiments, the food was supplemented with yeast and baked, with the addition of egg-yolk. The results were very good and were attributed to the yeast; lecithin was shown to be unnecessary. It may be seen that Röhmann's findings coincide with those on rats. Wheeler (356) carried out some experiments with mice, planned in accord with the first experiments of Osborne and Mendel on rats. Here, too, protein-free milk was used, and found insufficient as the sole source of vitamine; artificial protein-free milk proved to be entirely inadequate. It seems that mice need more protein than do rats in order to grow; yet the former grow more rapidly after the addition of a milk preparation than do the latter. MacArthur and Luckett (357) investigated the influence of lecithin, kephalin, cerebrosides and cholesterol as additions to an artificially prepared diet, and came to the conclusion that these substances are superfluous. Furthermore, they studied the influence of egg-yolk and found that the necessary substances (apparently vitamines, as they remarked at the time) are insoluble in ether, soluble in cold alcohol and thermo-stable. Butter and olive oil were inactive, without any other additions. Mitchell and Nelson (358) found that mice do not grow on an artificial diet till protein-free milk was added. From the foregoing, it is evident that mice behave like rats in their vitamine requirements.

Guinea pigs

Next to the pigeon and the rat, the guinea pig is one of the most widely used animals for the purpose of vitamine research, especially in the investigation of scurvy. It has been shown unsuited for other avitaminoses, though there are no lack of investigators who propose the use of the guinea pig for the study of pellagra and rickets, despite its demonstrated unsuitability. This animal is likewise little used for beriberi research although Schaumann (l.c. 2) reported, out of 65 guinea pigs fed on dry grain, two typical cases of beriberi, with subsequent histological demonstrations of multiple neuritis. These results have never been confirmed. On the whole, it is not easy to conduct planned feeding experiments with these animals, and

hence the difficulty of studying problems of nutrition. We know comparatively little of their vitamine requirements, particularly as to vitamines A and B, since in the study of experimental scurvy, dietary mixtures are used containing both of these vitamines. Hume (358a) has recently found that guinea pigs require vitamine A and hence these animals can be used for the detection of vitamine A in green foods but not in fats. Tozer (358b), in a pathological investigation of these animals could not find any difference between experimental scurvy and the changes produced by lack of vitamine A.

FIG. 30. LONGITUDINAL SECTION OF A RIB OF A SCORBUTIC GUINEA PIG, WITH FISSURES, HEMORRHAGES IN THE PERIOSTEUM AND PALE MARROW (HOLST–FRÖLICH)

We owe the discovery of experimental scurvy to Holst and Frölich (359) in 1907, although in 1895 a similar condition was observed in the same species by Theobald Smith (360). Holst and Frölich investigated scurvy in various animals and chose the guinea pig as most suitable for this purpose. It appeared to be unnecessary to choose a special diet, for these investigators noted that scurvy could be produced in guinea pigs with any diet that would do the same in man; this was also the case with any diet that was autoclaved at a high temperature. They recognized, with great perspicacity, that the symptoms were closely analogous to those in man. Guinea pigs

fed with rye, or wheat bread (and water), or with oats, rye, wheat, barley, and rice flour, died within a few weeks. Holst and Frölich used animals weighing about 350 grams since they could better withstand the great loss in weight (about 40 per cent). In a later publication, the same investigators (359) came to the conclusion that human, as well as guinea pig, scurvy may be attributed to the same causes; they sought also to differentiate between experimental scurvy and simple inanition. Starvation, in which there is a loss of from 30 to 40 per cent in weight, yields no scorbutic symptoms; on the contrary, scorbutic symptoms ordinarily appear before the animals show any appreciable emaciation.

FIG. 31. SCORBUTIC FEMUR OF A GUINEA PIG, WITH PALE MARROW AND ATROPHIC TRABECULAE (HOLST–FRÖLICH)

Not all workers accept the simple etiological explanation of scurvy in guinea pigs. Jackson and Moore (361) and Jackson and Moody (362) believed that there was an infectious factor in the etiology. Moore and Jackson (363) strengthened their belief after they had seen that raw milk could not protect these animals from scurvy. The explanation for this is to be sought in the probability that the milk used did not contain enough vitamine C for this type animal. In contrast to the above investigators, Givens and Hoffman (364) were able to demonstrate the absolute sterility of the blood of the diseased animals. Further, McCollum and Pitz (l.c. 69) believed that scurvy in guinea pigs was due to constipation, and made the sweeping statement that scurvy, as an avitaminosis, was non-existent, and that the known antiscorbutic properties of fruit juices could be explained by their laxative action. They stated that other laxatives, like phenolphthalein and mineral oil, could be used with the same success. These statements were further developed by Pitz (365) a co-worker of McCollum's, who found that lactose acted favorably, and that its function was to modify the bacterial intestinal flora. The observations of McCollum and his co-workers immediately aroused a storm of protest.[15] Shortly thereafter, Harden and Zilva (367) showed beyond a doubt that the results of McCollum were due to the fact that he gave the guinea pigs milk, ad libitum, and

[15] Torrey and Hess (366) have investigated the bacterial intestinal flora in guinea pigs and in infants, but failed to find any abnormalities.

in this manner prevented the onset of scurvy. When milk was omitted, lactose had not the least influence upon the progress of the disease. Later, these observations were confirmed by many others, and McCollum himself abandoned his view.

When we (368) spoke with doubt as to the identity of scurvy in guinea pigs and in man, it was based upon failure to obtain therapeutic results with milk, lime juice, potato and lemon juice. All of the difficulties mentioned by us at the time have been overcome by countless investigators, and at present there is no reason to doubt that in guinea pigs, we are actually dealing with scurvy.

As we have already said, the vitamine requirements of the guinea pig, aside from vitamine C, have been insufficiently investigated. Häusermann (l.c. 13) extended his experiments on the influence of iron salts on guinea pigs, without noting any results. Heim (369) found that guinea pigs could not live on raw or cooked cow's milk. Of eight animals, one died after a month, two after 2 months, and the other five remained alive for 3 months (the whole experimental period), but showed loss of weight. In another series of experiments, the animals died in from 4 to 14 days; the addition of cellulose was without effect,while malt extract and alcoholic extract of germinated barley exerted a definite favorable influence. Rondoni and Montagnani (370) conducted similar experiments with corn and oats, and with complete starvation. Guinea pigs on corn showed milder scurvy symptoms than those on oats. Of particular interest, is the work of Ingier (371) who investigated the influence of diet on pregnant guinea pigs. Here, too, a diet of oats and water showed scorbutic influence on the embryo. When this diet was used in the later stages of pregnancy, no pathological changes were found in the bones. A short feeding with the milk of the scorbutic mother, converted the latent scurvy into the active form. In pregnant animals, the disease developed more rapidly, with more marked symptoms, than in those that were not pregnant, and in the first stages of pregnancy lead to death. Ingier described a fatty degeneration of the nerves in these guinea pigs.

Conditions for the demonstration of experimental scurvy.—The foodstuff mostly used for the development of this disease is oats. According to the investigations of McCollum, Simmonds and Pitz (372), oats are poor in adequate protein, salts and vitamine A. It is undoubtedly true that under the working conditions maintained by

Holst, the guinea pigs soon lost their appetite and died, presenting mixed symptoms of scurvy and starvation. Therefore, it signified a step forward when, according to the procedure of Chick and Hume (373), the animals were given an addition of sterilized milk. This addition did not delay the development of the disease—the general condition of the animals being markedly improved—and corresponded with the view advanced by McCollum and his school as to the nutritive value of oats. The demonstration of experimental scurvy was no longer difficult, especially after the introduction of the milk addition, and investigations on this subject were easily carried out. For this purpose, Cohen and Mendel (374) used animals weighing from 110 to 250 grams. They believed that cod liver oil had no influence on scurvy, so that a complication of rickets was no cause for concern. Scurvy can be produced by feeding soya bean flour, which contains enough of vitamines A and B, supplemented with cellulose and salts. Hess and Unger (375) used oats, hay and water (to this may also be added cod liver oil) as a basal diet and found that guinea pigs weighing from 200 to 300 grams developed scurvy in two to three weeks.

In the Lister Institute in London, where many investigations on guinea pigs are being made, the original method of Holst and Frölich was modified so that the diet consisted of oats, bran and 60 cc. of milk for guinea pigs weighing 350 grams. The milk is autoclaved for one hour at 120°C.; on this diet the animals grow normally for about 15 to 20 days, after which the first symptoms of scurvy are noticed. The animals lose weight, and after 30 to 40 days they die of acute scurvy. In the presence of sufficient vitamine C, growth is uninterrupted.

The symptoms, already largely described by Holst and Frölich, are as follows: the molars become loose, with bleeding of the gums. Sometimes a bluish hyperemia is noticed and in rare cases, ulceration. Apart from this, hemorrhages are common in the soft parts of the knee joints and under the periosteum of the sternal ends of the ribs. Very often the connection between the ribs and the cartilage is loosened; there is a severing between the epiphysis and the shaft of the bones, especially the tibia. Later there occur duodenal ulcers, brittleness of the bones, hematuria and edema. In addition to these symptoms Chick and her co-workers (376) observed extreme pain and swellings of the joints, even in the first stages. The animal assumes

a characteristic posture ("scurvy position"), lying on one side, the painful limb stretched out into the air. In other cases, the animal lies with its head on the floor of the cage, a position caused by the pain in the jaws and gums ("scurvy face-ache position"). As soon as the teeth become loose, the animals refuse their food and death usually follows within the next few days. If the animals receive not quite enough vitamine C, they recover and begin to grow, but the joints do not become normal. An abundant addition of vitamine C before the beginning of the experiment has no effect on the time of development of scurvy, according to Hess (377); hence it would seem that the body has no reserve supply of this vitamine.

FIG. 32. "FACE-ACHE" POSITION OF SCORBUTIC GUINEA PIG (REP. MED. RES. COM.)

The post-mortem reveals rarification of the long bones, with fractures between the epiphyses and the bones themselves; fractures are also evident in the ribs. The bones show arrested ossification, and atrophy of the existing bone tissue and of the ossification areas; the bone marrow loses its lymphoid character at the diaphyseal ends, becomes poor in cells, and sometimes presents a homogeneous appearance ("Helles Mark"). Hess and Unger (378) called attention to their observation of rosary in scurvy, which might be mistaken for rickets. Rondoni and Montagnani (l.c.370) described pathological investigations of guinea pigs fed on corn. The symptoms seemed to resemble those of experimental scurvy although they were more mild. The organs mostly found altered were the spleen, thyroid gland and adrenals; the central nervous system, liver, digestive tract, kidney and bone marrow were slightly changed, while the myocardium and the lungs were still less affected. The changes in the thyroid and adrenals go hand in hand with a hypertrophy of the islands of Langerhans. Thyroid and spleen gave evidence of sclerosis; the adrenals, a diminution of lipoids; and in the cortex, atrophy and degeneration.

Rondoni (379) had already observed that the adrenals were somewhat enlarged, though containing less adrenaline. This signifies a great difference between scurvy and beriberi, for in the latter, the amount of adrenaline is increased. Rondoni believed that the picture of "maidismus" (corn-feeding) is different in scurvy and pellagra. McCarrison (380) studied the influence of a diet of oats and autoclaved milk. While the weight of the normal adrenals in guinea pigs is about 0.467 gram, that in scurvy was about 0.955 gram. Histological examination revealed hemorrhagic infiltration (which perhaps causes an enlargement of the gland) and atrophy of the cellular elements of the cortex and medulla. In spite of the size of the gland, the amount of adrenaline is diminished and has about half of its normal value. The quantity of adrenaline per gram of body weight dropped to about one-quarter of the normal. These symptoms arise before the other symptoms are apparent. LaMer and Campbell (381), working in Sherman's laboratory, were able to confirm the results of McCarrison completely. The thyroid gland, according to McCarrison (l.c. 295), may also be found increased in size, due to infiltration. Aside from this, McCarrison (382) found that the bladder had undergone pathological changes. Inflammation of the mucosa and the musculosa of the bladder and signs of degeneration of the epithelium were noted, associated with hemorrhagic infiltration.

As regards the metabolism in scurvy, we have the data of Bauman and Howard (383), differentiating it from simple starvation. The animal were fed on oats, and the inorganic metabolism was controlled. Nitrogen, sulphur and phosphorus elimination were decreased, sodium and chlorine were unaffected, potassium was retained, while the calcium and magnesium output was increased. These investigators observed the typical "white line" of infantile scurvy, noted by Fränkel. In comparison, there are the normal figures obtained by Smith and Lewis (384) with guinea pigs.

Through a determination of the alkali reserve of the blood of these animals, McClendon, Cole, Engstrand and Middlekauff (385) showed that scurvy of guinea pigs has nothing in common with acidosis. Funk came to the same conclusion somewhat earlier (l.c. 368), on the addition of sodium bicarbonate.

Lewis and Karr (386) found the blood and organs of scorbutic animals much richer in urea, as compared with those of the controls;

this could not be explained by hunger or loss of water. Karr and Lewis (387) studied the amount of conjugated phenols excreted. The figures obtained were normal and hence their conclusion, that in scorbutic guinea pigs there is an abnormal bacterial decomposition in the intestine, seems unjustified. According to McCollum and Parsons (387a), the prairie dog behaves not like the guinea pig but like the rat, in its vitamine C requirements.

Rabbits

Rabbits have been infrequently used in the solution of some of the vitamine problems. The reason for this is that these animals are much less affected by a lack of vitamines, which we have personally demonstrated (l.c. 368). We found that a rabbit may live on oats alone for more than three months, exhibiting individual variations in their behavior. The explanation for this may be found in a paper by Portier and Randoin (l.c. 217). In this investigation, rabbits were fed on cabbage and carrots, autoclaved for one hour at 125°C. The animals usually died after 11 to 17 days, with symptoms of an avitaminosis. When the experiments were repeated with a larger animal, these results could no longer be obtained; the animal was observed for three months during which time it gained weight. This observation found no explanation, till it was noted one day, that the animal was eating the feces. Two series of experiments were then arranged, in one of which the animals received, in addition to the diet, the feces of the other, kept also on the de-vitaminized food. The animals receiving feces were in good health after 100 days and gained weight. The investigators explained this result by saying that with the feces, bacteria were ingested which prepared vitamine for the animal by symbiosis. It must nevertheless not be forgotten, that feces may contain vitamine, as we shall see later on. Nevertheless, the observation of Portier and Randoin was of greatest interest.

Schaumann (l.c. 2) thought that he had produced beriberi in rabbits, fed on corn, which could be cured by yeast or Katjang-idjoe beans. Since corn is very rich in vitamine B, this condition was evidently confused with scurvy. Abderhalden and Lampé (l.c. 25) have observed paresis in a rabbit fed with rice, but beriberi has never been actually demonstrated in rabbits. On the other hand, scurvy in rabbits has been described by Holst and Frölich (l.c. 36), identical with scurvy in guinea pigs. In opposition to this, Morgen and

Beger (388) regarded the disease of rabbits fed on oats as an acidosis which could be obviated by the addition of calcium carbonate and sodium bicarbonate. With these additions, some of the animals lived up to 190 days, and often showed a gain in weight. We (l.c. 368) have repeated this work and have satisfied ourselves that the addition of alkali actually does excite the appetite and prolong life, but does not protect from death. McClendon, Cole, Engstrand and Middlekauff (l.c. 385) obtained the same results. Kurijama (389) found that the alkali reserve of the blood depends very much upon whether the food gives rise to bases or acids, although McClendon, v. Meysenbug, Engstrand and King (390) rightly observed that his results were complicated by scurvy, since he fed his rabbits on oats.

From what has just been said, we see that rabbits are hardly the proper animals to use for the study of scurvy. According to the work of Nelson and Lamb (391) rabbits seem to be adapted for the study of ophthalmia. Two rabbits, kept on commercial casein, dextrin, lactose, wheat germ, salts and an alcoholic extract of alfalfa grass, developed, after 60 days, an eye disease which they thought to be similar to ophthalmia; one of the animals was cured on the addition of butter.

Cats

Schaumann (l.c. 2) fed two cats on denatured meat heated in an autoclave with sodium carbonate at 120°C. One of the animals died after 42 days, the other, after 58 days with complete paralysis. It seems, according to the newer investigations, that what Schaumann saw was really beriberi. The experiments were repeated by Weill, Mouriquand and Michel (392) and they showed that the same symptoms could be obtained with cooked as well as with sterilized meat. While the animals died after 45 days on small amounts of fresh meat, nervous symptoms appeared after 35 to 39 days with sterilized meat. Voegtlin and Lake (393) produced typical beriberi in cats on fat-free meat, digested with 10 per cent sodium carbonate solution for three hours at 120°C. The beriberi symptoms were completely removed with vitamine B, sometimes after only 12 hours. The disease could be prevented by daily additions of 2 cc. of autolyzed yeast per kilo body weight, but not by the addition of 5 per cent butter or 10 per cent purified casein. On beef, heated without soda,

the animals remained healthy, except during pregnancy and lactation, when the vitamine requirements are greater. Osborne, Wakeman and Ferry (l.c. 102) found very little vitamine B in lean beef, and noted that cooking several times in water removes this substance, leaving only traces. Mackay (393a) and Tozer (393b) did not succeed in producing experimental rickets in cats in the absence of vitamine A.

Lions

Bland-Sutton (394) observed rickets in young lions in captivity when they are weaned too soon and fed on raw meat. Apparently, there is a lack of vitamine A, since the animals were cured with milk and cod liver oil.

Dogs

Dogs are now frequently used for vitamine studies and therefore we shall devote a little more space to this subject. The dog has become particularly important for experiments on rickets. In the historical section, we have already discussed the metabolism of dogs to some extent. It frequently happened, in these experiments, that the various investigators used a diet poor in vitamine although the purpose of the experiments was aside from the question under consideration. For instance, there is the work of Cahn (395) in 1886, who studied the results of chlorine hunger. Cooked meat was used as a food, and Cahn described a number of symptoms of chlorine hunger, among which was blood in the stomach contents, which might perhaps have been due to decreased capillary resistance. Similar experiments were also carried out by Trappe (396) with extracted meat, to study the significance of hydrochloric acid in the stomach. Rosemann (397) too, experimented with the same end in view, as did also Bönniger (398), using a diet of extracted meat. Bönniger observed that the animals refused the food and manifested vomiting, apathy and distress. It was indeed remarkable that these symptoms should have disappeared on the addition of sodium bromide. Pflüger (399) observed enteritis in dogs fed on sterilized meat, poor in fat.

A whole series of experiments, which have only historical interest at present, were made by Wolfgang Heubner (400) and his school (Lipschütz, Durlach) on the significance of phosphorus in dogs. The diet usually used for this purpose consisted of tapioca or white rice, egg-albumin, palmin, cane sugar and salts. Heubner thought

to obtain a phosphorus-free diet in this way, whereas in reality, he was dealing with a vitamine-free diet. Influenced by our work, Heubner later recognized the importance of the vitamines. From the work of Schmorl (401) who undertook the pathological examination of these dogs, it may perhaps be noted that Heubner was dealing with a mixed avitaminosis. In young dogs, rachitic symptoms prevailed, although it is not clear whether the bone changes were of scorbutic or rachitic origin, or due to a combination of both.

Still less satisfactory were the investigations of Masslow (402), who studied the same problem as Heubner, but on dogs, and on practically the same diet. Although the work was done in 1913, Masslow evidently knew nothing of the significance of the vitamines. His only observation of interest to us was the general emaciation of the animal body on a diet actually free of vitamines.

In discussing vitamine problems, we must note, first of all, that dogs seem to be sensitive to a lack of all three vitamine types. Nevertheless, although Holst and Frölich (l.c. 36) produced experimental scurvy in dogs, they seemed to be least sensitive to a lack of vitamine C. Schaumann (l.c. 2) fed dogs on denatured meat and described a condition similar to beriberi, with paresis and changes in the pharynx and tongue; the latter appears to be more likely of a scorbutic nature. Despite this, Schaumann (403) held that all these symptoms could be removed by administering yeast, and since yeast contains no vitamine C, he must have been dealing with beriberi. It should be pointed out here that the findings on beriberi in dogs are not concordant. Theiler, Green and Viljoen (l.c. 278) stated that they could not produce beriberi or scurvy in dogs by feeding with white rice; the animals merely suffered from malnutrition. Voegtlin and Lake (l.c. 393), on the contrary, described true beriberi in dogs fed on meat, extracted with soda.

Karr (404) fed dogs on lard, cane sugar, salts and protein, in the form of casein or wheat gluten. After 3 to 9 weeks, the animals refused the food. It was noted that grown dogs needed very little vitamines A and C, since yeast alone, in amounts of 0.1 gram daily, restored the appetite to normal, while an addition of 0.2 gram cured beriberi in 8 to 12 hours. The utilization of protein seemed uninfluenced by the presence of vitamine B.

Proceeding to the rachitic-like disease in dogs, we come first to the work of Stilling and v. Mering (405) in 1889, who fed dogs on

extracted meat and fat in order to study osteomalacia and the effect of a calcium-poor diet. The experiment lasted 126 days. Hebrant and Antoine (406) described a typical osteomalacia in dogs. Guérin (407) observed rickets in young dogs fed on meat, while animals from the same litter, which were suckled, remained unaffected, Some of these experiments were made not only with a vitamine-poor but also with a calcium-poor diet and the pathological condition described was nothing but osteoporosis. To this category, belongs the work of Roloff (407a) in 1879, and also the communication of Reimers and Boye (407b). Bull (408) described a severe occurrence of rickets in young dogs fed on cooked meat and pollard, in Adelaide, Australia.

However, the systematic investigations of Mellanby (l.c. 95) are of chief interest in this chapter. This investigator fed young dogs 5 to 8 weeks old on diets of various composition, poor in vitamine A. These diets were more or less suitable for the production of rickets and in the end, the following were chosen for this purpose.

	DIET A	DIET B
Skimmed milk	175 cc.	250–350 cc.
Wheat bread (white)	ad libitum	ad libitum
Linseed oil	10 cc.	10 cc.
Yeast	10 grams	5–10 grams
Orange juice		3 cc.
NaCl	1–2 grams	1–2 grams

Some 200 dogs were used in this work; at first, they were kept on the diet for 6 months but later, a shorter time, for it was demonstrated that dogs on diet B developed rachitic symptoms after 6 weeks. Mellanby investigated the behavior of various foodstuffs of animal and vegetable origin, and found that those that are protective against rickets are rich in vitamine A. Still, meat poor in fat had some effect in delaying the onset of rickets. Quickly growing dogs develop rickets soonest, which apparently shows that the more active the growth the more vitamine A is necessary.

Symptomatology. The ossification of the bones proceeds in an abnormal manner; hence the bones bend and the ligaments become loose, making the deformities even more apparent. Swelling of the epiphyses of the bones was easily noticeable and according to the severity of the condition, deformities of the thorax developed with a

Fig. 33. Rickets in a Dog (Mellanby; Rep. med. Res. Com.)

Fig. 34. X-Ray of Bone of Rachitic Pup (Mellanby; Rep. Med. Res. Com.)

typical rosary. The animals were very little resistant to infections, were apathetic, lethargic and extremely unwilling to exert themselves, at a time when the existing bone changes could not yet have been responsible. This was undoubtedly due to the impairment of the muscular tonus.

After death, the bones were found to be very poor in calcium and showed, in the X-ray, an appearance characteristic of rickets, as may be seen in the illustration. Of two dogs of the same litter, one animal received only the "rachitic" diet, the other, vitamine A in addition. The malformation of the cartilage epiphyses and the more frequently occurring osteoid tissue are the two chief symptoms. According to Henderson (408c) the muscle of the rachitic dog contains less creatine than the normal. Findlay, Paton and Sharpe (408b) conducted metabolism experiments on rachitic dogs. A calcium-poor diet caused osteoporosis, but not rickets, while in rachitic dogs the blood and the tissues showed the normal calcium content.

The significance of Mellanby's experiments for the study of rickets will be discussed further in the description of this disease.

In conclusion, we shall mention an investigation of Chittenden and Underhill (l.c. 28). They fed dogs on cooked peas, zwieback and cottonseed oil, and noted a disease which they believed to be analogous to pellagra in man. The disease developed after 2 to 8 months, showing that a larger amount of peas was less harmful. The disease, which could be cured by adding meat to the diet, produced ulcerations of the mouth; in particular, the entire enteron was hemorrhagic. Besides, there was observed a great loss of weight, peculiar gait and diarrhoea. If a part of the peas was substituted by casein, the symptoms appeared sooner. This shows, perhaps, that the condition is associated with a lack of vitamines, since peas contain some vitamine but not enough. To our knowledge, this work has not yet been repeated by any other investigator.

Domestic animals

We come now to the consideration of domestic animals, among which we shall discuss goats, sheep, horses and pigs. In this field, there are questions which are not yet quite clear, but which possess a definite significance in their social and economic aspect. We have very little accurate knowledge of the vitamine requirements of these animals. In various parts of the world, we encounter diseases,

which, though not yet explained etiologically, nevertheless always give some indications showing that they may be avitaminoses. In most cases, they occur on a large scale in Nature, and consequently are specially deserving of our interest.

Sheep and goats. Experimental investigations with these animals were first made by Schaumann (l.c. 2). A goat, fed on corn and white rice, lost weight rapidly and showed, according to Schaumann, paresis, although corn certainly contains sufficient vitamine B. The animal was then given yeast, Katjang-idjoe beans, and green fodder (the latter for one month), with a resultant gain in weight. This effect was attributed to the yeast and the beans, although the green fodder very likely played a greater rôle. This experiment was not clear and consequently is of little significance. Henriques and Andersen (409) administered parenterally hydrolyzed meat, glucose, sodium acetate and salts to goats. For 20 days, a marked nitrogen retention was noted. They then heated the food mixture for 20 minutes at 110°C., and were able to show that it was no longer possible to maintain the nitrogen balance, except when the heating was not carried beyond 100°C. These results were attributed to the susceptibility of tryptophane to heat. Fingerling (410) fed lambs on inorganic and organic phosphorus combinations, without observing any particular differences. He made the correct observation that the frequently noted improper nutrition with inorganic phosphorus compounds was not due to the character of the phosphorus, but to the composition of the food itself.

Theiler, Green and Viljoen (l.c. 278) were unable to demonstrate an avitaminosis in sheep and goats. These animals tolerated the rice diet better when an addition of autoclaved hay or straw was made. Very soon, enteritis made its appearance, though in the opinion of these investigators, this had nothing to do with the diet. At all events, the requirements of vitamine B appear to be very modest.

The naturally occurring nutritional disturbances were described by Hoare (411) as a form of edema, which develops because of an insufficient fodder and poor climatic conditions. A disease of sheep, known as "staggers," was described by Jones and Arnold (412) in South America. It is a nervous disturbance of sheep, as well as horses and cattle, caused by the consumption of coarse grass (Poa Argentina). They were not clear as to the etiology, and were more inclined

towards the conception of a toxic phenomenon. The observed symptoms were general weakness, muscle tremors, peculiar movement of the head, stiffness of the limbs, intermittent paralysis with spastic appearances, particularly after irritation, impairment of the visual capacity and conjunctivitis. The disease could be produced experimentally by feeding pampas grass. The time necessary for this varied between 2 and 21 days, averaging 10 days, during which spontaneous cures often occurred. By changing the diet, it was possible to effect a cure. Young animals were far more susceptible than grown ones, and the disease was attributed to a poison in the grass, present at all seasons and in all parts of plants. The post-mortem revealed nothing positive and afforded no definite indications of the nature of the toxin. Reid and Aston (413) observed a bone cachexia in sheep, which they referred back to a lack of inorganic factors of the diet.

Reindeer. Ibele (414) noted a disease called "Lecksucht" supposed to be due to the lack of alkaline earths.

It is difficult, without having personally seen these animals, to draw a picture of the nature of the disease. The observation that in one case the disease could be produced after two days feeding, would speak against its being an avitaminosis, if the investigators had not explicitly emphasized that this is possible only in certain susceptible animals. This lack of resistance can be explained, at least theoretically, by a previously occurring deficient dietary.

Pigs. The nutrition of these animals was studied from two viewpoints—first, from that of the animal breeders in connection with the food requirements, and secondly, from that of laboratory men in relation to the vitamines. It is true, as Evvard (415) pointed out, that if these animals are permitted to select their own food, their natural instinct will lead them to choose a correct diet. Conditions are different in winter, however, when a prepared diet must be provided. Hart and McCollum, as well as their co-workers, studied the exact food requirements of pigs in relation to growth, maintenance and reproduction. Such studies were published by Hart, McCollum and Fuller (416) in 1908. They showed that pigs fed on rice and washed bran suffered from osteoporosis, while those fed on unwashed bran did not manifest this disease. At that time, the conclusion was made that the phosphorus compounds were washed out with water—which of course does not hold today. Hart and McCollum

(417) observed that between wheat and corn, there are great differences as regards the growth-promoting properties. While the animals attained a weight of 100 pounds on wheat, on corn they weighed from 200 to 300 pounds. McCollum (418) fed young pigs on wheat, oats and corn and found that casein, as well as skimmed milk, was a better source of nitrogen. After three months, the animals declined and McCollum left the question open as to whether this condition was due to lack of salts or vitamine, or to the presence of a toxic substance. Hart and McCollum (419) could not keep young pigs alive for a long time on corn and gluten, while the addition of casein or milk appreciably improved growth. Hart and Steenbock (420) investigated a series of plant products, with the addition of the same animal foods as above, and came to the conclusion that rice, wheat, corn, potatoes, and cabbage possessed little nutritive value, but it was not further investigated whether the vitamines played a rôle. Later, Hart and Steenbock (421) sought to settle the question as to whether the presence or absence of vitamines has any effect. For this purpose, grown pigs were kept for a long time on corn and oats. Eventually, symptoms of the disease appeared, such as stiffness of the legs and difficulty of getting about, these conditions being specially manifested in pregnancy and lactation. A great number of young were born dead. If these symptoms were not noticed in the first litter, they were present in the second. In most cases, an improvement was seen when the cereal diet was substituted by a corn-alfalfa mixture, which is much richer in vitamines. The dietary deficiency was however attributed largely to the unfavorable composition of the inorganic constituents. These exact experiments are particularly important for the recognition of the etiology of some very frequently occurring diseases of cattle which are of practical importance, and which have lately been quite often attributed to the effect of a poison. We see, however, that feeding in the stable may lead to dietary deficiencies, without it being necessary to assume a toxic cause.

The superiority of skimmed milk over all other food mixtures was also set forth in a number of publications of Klein (422). This favorable influence may be explained either by a better utilization of the protein, by the vitamine content, or by both factors.

Despite all these investigations, and still others to be mentioned, we are not yet accurately informed as to the vitamine requirements of

pigs. This is so because few investigations in which only the vitamine factor is lacking, while all other factors are controlled, have been carried out. All of the above-mentioned investigations are complicated by mixed symptoms, and it is therefore impossible to form an unbiased opinion as to the nutritive requirements of these animals. This is all the more regrettable, since the pig, which eats anything, is peculiarly adapted for the study of human nutrition.

In 1907, Holst and Frölich (l.c. 36) described, in rice-fed pigs, symptoms which were thought to be mixed forms of beriberi and scurvy. Since white rice is lacking in all three vitamines, it is impossible to say which one is most important for these animals. Theiler, Green and Viljoen (l.c. 278) on the contrary, could observe no specific symptoms on this food. In one case, gall stones were found; in others, enteritis was noted, which may perhaps be regarded as a sign of an avitaminosis. Plimmer (422a) observed scurvy in pigs fed on cooked food, the symptoms of which were relieved by the administration of uncooked food. Green (423) carried out investigations with a diet poor in calcium, and in this way found two kinds of undernourishment; one kind depends upon protein which was qualitatively shown to be inadequate, while the second could be attributed to insufficient protein and salts addition. The animals were in poor nutritive condition and showed defective bone growth with insufficient calcification. Substitution of lard by butter exerted no favorable influence. It is one of the few investigations on the nutrition of domestic animals, in which vitamine A was given special attention. Another investigation, in which the presence of vitamine A in the diet was provided for, was that of Hart, Miller and McCollum (424) A typical composition of the diet used by them was 95.5 parts wheat meal, 2.5 parts wheat gluten, 2 parts butter, di-potassium phosphate and calcium lactate. On this diet, the animals developed symptoms quite similar to those of beriberi. Among other symptoms, pathological changes were noted in the spinal cord which showed fatty degeneration. The symptoms made their appearance after about 9 months, when a decrease in weight, difficulty of forward motion, rough coat, labored breathing and muscle tremors were observed. There was also stiffness of the extremities and dragging of the legs; if the animals were helped to their legs they again sank at the knees. Since McCollum, at this time, denied the existence of vitamine C, the diet used was considered as complete, and the

disease was attributed to toxic action of wheat. Addition of whole milk had only a slight effect upon the general well-being of the animals, but the addition of alfalfa grass soon permitted of the development of a normal condition. In all these investigations, growth was interrupted when the general condition of the animal became worse. It seems to us that without further indisputable evidence, it is difficult to accept the view as to the toxicity of wheat kernel. It may be possible that food mixtures were used, the vita-

FIG. 35. BERIBERI-LIKE DISEASE IN A PIG ON A DIET CONSISTING OF 45 PARTS CORN, 45 PARTS OATS, 10 PARTS OIL SEED PRESS-CAKE AND 5 PARTS BUTTER (HART, MILLER AND MCCOLLUM)

mine A content of which was barely sufficient, while vitamine C was almost completely lacking. Experiments with the addition of these vitamines were not made.

With products derived from cotton seed, the behavior was naturally different. These contain a toxic substance called "gossypol." Such products, fed to pigs, cause death in 50 to 80 days, according to Roberts (425). But even in these cases, there seems to be a vitamine deficiency, according to Rommel and Vedder (426). In 8 to 15 days, this food produces in these animals a picture of disease resembling

perhaps the wet form of beriberi in man, and quite analogous to the symptoms which pigs develop when fed white rice. Zilva and collaborators (426a) found that the pig was susceptible to the development of experimental rickets, but in this animal, the lack of vitamine A (of milk) was not the sole causative factor.

In spite of this series of results we are not yet clear as to the vitamine requirements of pigs, so that further work is necessary.

Horses. In 1908, Friedberger and Froehner (427) described edema in horses and oxen which performed heavy work in the sugar refineries, and were fed on sugar beet residues. These residues contained only a slight amount of protein, about 0.5 per cent, very little dry substance (5 per cent) and high water content (95 per cent). This diet which, aside from the protein, appeared to be lacking in other constituents, together with the hard work and high water content of the food, was supposed to be responsible for the development of this disease. Similar pathological conditions were likewise noted in horses by Hutyra and Marek (428).

Scheunert, Schattke and Lötsch (429) observed a disease in horses, resembling osteomalacia, in the poor mountainous districts in Saxony. Kawakami (430) observed in horses, a disease called "sukumi" or "gokusukumi," which developed when rice, barley and oats were fed. The disease exhibits a certain similarity to experimental beriberi and starts with gastro-intestinal disturbances. Theiler, Green and Viljoen (l.c. 278) fed horses on white rice for six months without noticing any signs of an avitaminosis. This is the more remarkable since white rice is supposed to be lacking in protein, salts and vitamine A, besides vitamine B.

Cows and oxen. In 1913, the late Dr. Donald Macauley called our attention to a South African cattle disease, known there as "Stijfziekte" and "Lamziekte." On perusing the available literature, it is apparent that similar conditions are known in all parts of the world. Stewart (431), for instance, reported a disease known in Australia mostly as "rickets."[16] In the poor mountainous districts of Saxony, according to the report of Lötsch (432), there is not so infrequently a pathological condition called "Stallmangel," supposed to be caused by a diet poor in salts. This severe disturbance of metabolism reminds one strongly of human rickets and osteomalacia.

[16] Stewart was disposed to consider the disease as an avitaminosis.

From New Zealand, there was a report by Aston and Reakes (433) on progressive anemia, known locally as "bush-sickness." It was supposed to be caused by lack of salts, and differed from lamziekte. In addition to these naturally occurring pathological conditions, we have the experience gained by the experimental feeding of cattle. Russel and Morrison (434) found that cattle fed on oats, straw and butter, or casein, butter and oat-straw, gave birth to very miserable looking offspring. This condition was not regarded as an avitaminosis but as a lack of calcium, since an addition of these salts seemed to improve the condition of the animals. Fleischmann (435) noted that calves fed on hay often became sick, and he proceeded to study the chemical changes attending the drying of the grass. He showed that lecithin and phosphoproteid, as well as protein, underwent some decomposition during the process of drying. However, since the total nitrogen showed no change, we may assume that there was no loss of inorganic constituents. The real difference observed must be looked for in the vitamine content.

We find a somewhat different conception in the work of Henry (436). In the description of an Australian cattle disease known as "impaction paralysis," resembling lamziekte, he attributes the cause of the disease to the poverty of the soil in calcium and phosphorus. The disease appears at the end of the dry season, in places where the vegetation is injured by the Australian rabbit-plague. The disease was found where there was an abnormal desire to eat bones and where osteomalacia had also prevailed for some time. A favorable effect was noted on adding a nourishing diet, such as skimmed milk. We have already mentioned the interesting assumption of Davis (l.c. 195), who held that in the last analysis the poverty of the soil was the cause of diseases of domestic animals. When the vegetation is so impoverished through extraneous conditions that it no longer provides sufficient calcium and phosphorus for the animals, it is conceivable that other important substances, for example, vitamines, might also be lacking. It must likewise not be forgotten that in the avitaminoses there is frequently a loss of inorganic constituents, and this condition might manifest itself in an abnormal desire for bones or other materials rich in calcium and phosphorus. For example, Forbes (437) stated that domestic animals often display an abnormal craving for mineral substances. Pigs and cattle sometimes eat large quantities of bone meal, with resultant improvement in

health. Cattle growers know that the condition of the legs in horses depends upon the fodder and largely on the nature of the soil. Place (438) believed he saw among cattle not only diseases similar to beriberi but also scurvy-like conditions in cattle, horses and sheep, infantile scurvy in calves and sheep, and diseases of the type of pellagra, with skin and gastro-intestinal symptoms.

Stijfziekte and lamziekte. These diseases of cattle and goats occur widely in South Africa, and are described by Theiler (439). These conditions, particularly lamziekte, have become of such economic importance that a great number of well-known investigators have occupied themselves with this problem. In some severely affected districts, the lamziekte problem has become so acute that it no longer pays to breed cattle because of the great mortality. Of the two diseases, stijfziekte has been least investigated, apparently because of its lesser practical importance. It is a bone disease of young animals, accompanied primarily by swelling of the joints, the metatarsals and metacarpals, and secondarily, the epiphyses of the long bones. These swellings are painful and since the fore-legs are most affected, the animal tries, as far as possible, not to use them, and runs around with forward-bent hind legs and hunched back. Muscular weakness and abnormal appetite (pica) are among some of the symptoms of the disease. The temperature remains normal. The diaphyses of the diseased bones, on sectioning, appear deep red and blotched with bloody-serous liquid.

FIG. 36. STIJFZIEKTE (THEILER)

Some forms of stijfziekte resemble lamziekte very much, without necessarily indicating a relationship between the two, in the opinion of Theiler, Green and Viljoen (l.c. 278). Theiler admitted that stijfziekte might be an avitaminosis. If this were so, it would help clear up the problem of lamziekte, since it could serve as a proof that the nourishment of cattle in some sections of South Africa could be improved. As it happens, stijfziekte may be cured by a change of

diet, according to available data. Feeding of good grass, green barley and green millet is supposed to be accompanied by beneficial results.

Lamziekte or gal-lamziekte. The history of lamziekte research is particularly instructive since it shows us how easy it is to go from one extreme to another. In times of great popularity of the vitamine theory, one is tempted to classify every condition that bears some analogy to the already known avitaminoses in the same group. Lamziekte, according to the present stage of investigation at least, is not an avitaminosis and would not have found a place in our treatise if it were not for the possibility of explaining one of the etiological factors through a lack of vitamine.

This disease is prevalent in all breeds of cattle without regard to species or sex. Young, pregnant or milking animals appear to be specially susceptible. Lamziekte occurs particularly after long periods of draught, and is manifested by paralysis and contractions associated with the degeneration of the peripheral and central nervous system, roughly resembling avian beriberi. We note here paralysis of the legs, opisthotonos, dysphagia and paresis of the tongue. Lack of appetite and pica (abnormal craving), excessive salivation, loss of milk, constipation and also diarrhoea—all these constitute the earlier symptoms. The temperature appears to be subnormal. The usual progress is sub-acute and lasts some weeks; the animals sometimes show an apparent improvement, but fatal results ensue when a second attack occurs. In spite of a cure, the contractions may still persist for a long time. Acute forms last from two to seven days, and yet sudden developments are noted, in which apparently healthy animals fall into a comatose condition in 10 to 20 hours, which, accompanied by sub-normal temperature, results in death. This condition has been frequently confused by South African farmers with another called "poverty," and resembling malnutrition. This condition, "poverty," may eventually prove to be a concealed avitaminosis.

Pathological anatomy of lamziekte. The findings have been specially described by Hedinger (440). They consist of hydrothorax, and ecchymoses of the pleura, epicardium, endocardium and thymus; hyperemia was observed in all the abdominal organs, in the mucosa of the fourth stomach, intestines and lungs; a moderate hydropericardium was frequently noted; enteritis of the small intestine, coupled with hemorrhages and ascites, with a dilatation of the heart,

occurred not infrequently. In the bones, on the contrary, at least microscopically, no changes were found.

Etiology. We may well imagine that because of the great practical significance of this disease no effort has been spared to discover its cause. One of the older theories of Theiler (l.c. 439) assumed the presence of toxins in the grass. This theory was experimentally tested by Viljoen (441) in that he fed a series of animals on various kinds of grass, found in regions affected by lamziekte, with negative results. The theory of infection was tested by Spruell (442); cows and goats received intravenous injections of the blood, lymph glands and ascites fluid of sick animals; here too, the experiments proved to be negative. Mitchell (443) and Walker (444) sought to bring about the disease in healthy animals by using carcass material of lamziekte animals, but in vain. We formerly held that lamziekte was similar to beriberi, but this view was modified by us in 1915 (445), in that we considered it to be similar to rickets. This view is also held by Hart, Steenbock and Hoppert (445a), who arrived at this conclusion from experiments with goats. Here cod liver oil had a distinctly favorable action on the calcium metabolism. In spite of this, our first opinion was carefully tested, and Stead (446) attempted to show that lamziekte was analogous to beriberi. He fed pigs on meat obtained from animals which had died of lamziekte, in the belief that it was poorer in vitamine B than normal meat. Cattle were also fed on white rice, samp (a product made from decorticated corn), white flour and oats; they showed pica, stiffness of the legs and pains on walking. Theiler, Green and Viljoen (l.c. 278) have investigated a series of animal species, already mentioned previously, as to their vitamine B requirements; horses, dogs, goats, sheep and pigs were used. They were kept on a diet consisting chiefly of white rice, and in no case was there any symptom characteristic of beriberi. Cattle were kept on white rice for a year without the appearance of any beriberi symptoms. In the worst case, symptoms were noted, in accord with the findings of Stead, which were thought of as laminitis and which could not be attributed to a lack of vitamine B. Latterly, Stead seems to have abandoned his view in favor of Theiler's.[17] One cow fed on white rice and autoclaved straw or

[17] Theiler said that no grass could be so poor in vitamine as was the diet which he used and he believed, therefore, that it had nothing to do with an avitaminosis. The survival of the animal, he attributed to a bacterial symbiosis (p. 75).

hay, gave birth to a calf that was blind. The validity of the vitamine hypothesis was tested in two ways. First, through a repetition of Stead's experiments, by feeding to healthy animals the meat of animals which had died of lamziekte, definite indication as regards impoverishment of vitamine B could not be found. Secondly, the influence of substances rich in vitamine B was studied. An addition of orypan (extract of rice polishings) and yeast had no effect, and we may therefore assume, with certainty, that lamziekte cannot be caused by a lack of vitamine B. Experiments with other vitamines were not tried.

In the meantime, Theiler and his co-workers (447) have apparently completely cleared up the etiology of lamziekte. They maintain that in the etiology of this disease, six phases prevail, which are responsible for its occurrence, and are related to each other, like the links of a chain.

1. A toxin or poison which causes the disease.
2. Toxin-forming saprophytes.
3. The availability of carcasses from which the toxins are formed.
4. Pica, or abnormal craving, which leads animals to eat carcasses, not present under normal conditions.
5. The character of the vegetation and the soil (and climate) upon which pica depends.
6. Susceptibility of the animals to the toxin.

The toxin was systematically investigated and seemed to be similar to the botulinus toxin. It is very active, inasmuch as 0.00001 cc. per kilo body weight is a fatal dose. Lamziekte may also be produced experimentally by administration of this toxin. The prophylaxis and therapy consist of the elimination of one of the six above mentioned links of the chain, at least theoretically. Practically, however, it seems that the best starting point is the pica and since this condition is of greatest interest to us, we shall speak of it in greater detail.

Pica. It has been demonstrated in South Africa, that not only cattle may exhibit this condition, but also ostriches, goats and poultry. Pica is supposed to be a nervous ailment, which arises only where the condition of the soil is such that the water quickly passes through the upper layers. Because of this, the soil becomes poor in phosphorus and hence produces a vegetation poor in this element. Pica itself is not dangerous, but becomes so when the

animals find infected carcasses to eat. This condition is tested by giving the animals rotten bones; if they chew these bones, then the diagnosis of pica is verified.

Cure of pica. To overcome pica, a mixed diet may be used which will cure the disease in one month. Two pounds of wheat bran cures it in three weeks, and 112 grams of bone meal is supposed to have the same effect. Besides this, the addition of calcium phosphate and sodium phosphate, and even phosphoric acid in drinking water, may be used.[18]

Etiology of pica. As we have seen above, Theiler thought that the chief cause of pica was the poverty of the diet in phosphorus, accompanied perhaps by too much calcium, although it is admitted that other dietetic factors also may play a rôle. Theiler also stated that on analysis of the diet, the phosphorus was found within the limits of the accepted standards for such animals. He concluded therefore, that the accepted figures had to be revised. We know from the study of deficiency diseases, that under such conditions there is often a negative calcium and phosphorus balance, and it has been stated from time to time, that the addition of phosphorus (we refer only to phospho-cod liver oil, hypophosphites, etc.) may result in a definite improvement. This may be the case with pica. The animal perhaps has sufficient phosphorus in the food but is unable to retain it because, we may say, the diet lacks vitamine A. An addition of phosphorus, in whatever form, may perhaps lead to a temporary improvement and, in the light of the modern conceptions of lamziekte, may also protect against this disease, since the animals would not eat the carcasses. But the cause of pica and the metabolic disease may endure till the arrival of a better season and a recovery of the vegetation. For it can be shown definitely that pica prevails only at certain times of the year, when the vegetation thrives but poorly. If this is so, then vitamines, as well as salts, may be lacking; a normal vegetation should provide everything for the animal that is necessary to life. The simultaneous development of undernutrition, in the form of "poverty," and perhaps also stijfziekte, in lamziekte regions, is illustrative of the profound dietetic deficiency of the local vegetation. As to the etiology of pica, this can be cleared up only by exact investigation, especially by metabolism experiments. Such accurate

[18] In this connection it is interesting to compare the influence of phosphorus on pica with that on experimental rickets in rats (p. 327).

investigations appear still to be lacking. Above all, it must be determined, in the event that the disturbance of metabolism may be cured by the addition of phosphorus, how vitamine A and C, which may well be present in poor vegetation in sub-optimal amounts, would act. The influence of these vitamines on pica has been very little investigated.

Monkeys

Only the lower types of monkey have been used of late for vitamine studies; the first work was that of Schaumann (l.c. 2) who fed monkeys on washed white rice, with the result that the animals died in 74 days. Before death, there was paresis of the hind legs, the appetite had disappeared and the animal showed a 27 per cent loss in weight. The histological nerve findings were not so typical for experimental beriberi, and controls with the addition of vitamine B were lacking, to prove that this condition really was caused by lack of this substance. Shiga and Kusama (448) have described a real beriberi in monkeys, with anasarca, hydropericardium and edema of the lungs, while Noë (449) was unable to induce beriberi in these animals by feeding rice. McCarrison (450) tried then to produce beriberi in Macacus sinicus, by feeding autoclaved, ground white rice, while in another experiment extra butter was added. The first group died in about 23.4 days and the second (with butter), after 15 days. None of the animals lived longer than 100 days. Although typical beriberi or edema was not noted, the clinical symptoms consisted of progressive anemia, gastro-intestinal disturbances and progressive asthenia. Stomach, intestinal walls and omentum were very thin and had lost their fat content. Congested, necrotic and inflammatory changes were apparent in the entire gastro-intestinal tract. Aside from this, there were degenerative changes in the neuro-muscular apparatus of the intestines, leading to the distention of the stomach and to other parts of the intestine. Signs of necrosis were seen in the secretory layers, and attenuation of the elements protective against infection was noted. These findings need not of course be attributed only to a lack of vitamine B, since in the diet utilized, vitamine C was also lacking.

The monkey is of greater value in the study of scurvy, as was found by Hart (451), and later by Hart and Lessing (452). Young monkeys kept on condensed cooked milk, rice and white bread,

developed a condition which resembled infantile scurvy in its symptoms, while in grown monkeys, the symptoms were more like those in grown people. In their book, they gave a detailed enumeration of the symptoms and the pathological findings (with many illustrations), so that we must refer the reader to this book for greater details. Particularly were the changes in the bones accurately described. The experiments of Hart were confirmed by Talbot, Todd and Peterson (453), and a few years later by Harden and Zilva (454). The latter produced scurvy on a diet containing only traces of vitamine B, but concluded that in future investigations, it would seem desirable that the diet should contain sufficient vitamine A and B. In our opinion, too, this is very important, since in vitamine studies, it must be so arranged that only a single factor is lacking. Harden and Zilva were able to cure the disease by means of antiscorbutics. In a later publication, they (455) showed that it took two months to develop scurvy in monkeys. The diet used by them consisted of 300 grams of rice, 50 grams of wheat germ, 2 grams of salts and 5 grams of butter. A daily administration of 2 cc. orange juice per os sufficed to protect the animals against scurvy. If smaller doses were given, the animals developed scurvy, with paralysis of the hind legs, which appeared to be a pseudo-paralysis. It is interesting to note that the monkey requires less vitamine C than does the guinea pig. A monkey weighing from two to three kilos needs as much as a guinea pig weighing 300 to 400 grams.

Among other symptoms, Hart and Lessing (l.c. 452) noticed an exophthalmus on the left side, with severe hemorrhagic edema of the upper eye-lids. The same observation was also made by Zilva and Still (456).

Chemical studies of the mineral metabolism of the scorbutic monkey were made by Howard and Ingvaldsen (457). In this case, the disease was produced with condensed milk in 4 months. Loss of salts, usually so marked in man and guinea pigs, was not noted here.

Harden and Zilva (458) reported an experiment on three monkeys, two years of age, fed on 250 to 300 grams of rice, 10 grams of yeast extract (marmite), 2 grams of salts and 4 cc. orange juice. One of these animals received no fat and no vitamine A, and developed an edema with diarrhoea in 289 days. The second animal received butter and lived 299 days, whereupon the experiment was interrupted.

The third animal was given olive oil, and died after 262 days without characteristic symptoms. Butter had no growth-promoting influence. Since the diet was very poor in fat, and since the significance of fat for monkeys is not known, the investigators wished to await further results before saying what rôle vitamine A plays in the development of this edema. However, Hewlett and de Korté (459) stated that in 1907 he had described edema in monkeys which had received sufficient milk to provide for their vitamine A requirements; nevertheless, he might have been dealing with a lack of vitamine B. No albumin was noted in the urine of these animals.

In conclusion, we wish to point out that Chick and Hume(460) have described, in a monkey, a condition which might be considered to be identical with human pellagra. The diet used consisted of sugar, corn starch, salts and corn gluten, in varying amounts, so that in this way more or less protein was given. All of the three known vitamines were present in the diet. We shall speak again of this experiment in discussing the etiology of pellagra.

For the investigators who are interested in nutrition experiments with these animals, it may be of interest to point out how monkeys are raised by dealers and fed in our zoölogical gardens. Schaumann (l.c. 2) states that in the Institute for Ship and Tropical Hygiene in Hamburg, monkeys are kept on a porridge made of cooked white rice; in addition they receive carob seed, peanuts and fruits. On personal inquiry at the New York Zoölogical Garden, we learned that the monkeys were given cooked rice, a cup of milk, bananas, apples, sweet potatoes, onions and carrots; once a week they were given some powdered sulphur. The chimpanzee received two raw eggs in milk, bananas, two oranges and a dozen cooked prunes every two days. The monkeys lived for years on these diets in very good health.

Vitamine requirements of man

After having discussed the vitamine and food requirements of various animals, we are prepared to study the above relationship in man. It is not infrequently seen that clinicians are unwilling to apply the results of animal experimentation to man, and they are justified in being sceptical. Yet we must emphasize that all we have discussed in the preceding chapters is based upon a solid foundation. We are dealing here with the fundamental laws of cell nutrition

applicable to all living organisms. As little as the modern clinician can afford to ignore the physiological significance of the proteins or of certain amino acids, just so little need he doubt the importance of the vitamines for human nutrition. It is true that the conditions leading to deficiency diseases in man are perhaps not so clear cut as in animal experiments, since in the latter, we may choose such conditions as will permit of the study of the significance of a single constituent exactly. In spite of this, we do not yet know all there is to be known of the laws of nutrition. It has been frequently necessary to proceed empirically; for example, we fed white rice to chickens and pigeons without knowing at the time that this foodstuff lacks other important constituents besides vitamine B. We were still in the empirical stage when we wrote our first edition, but since then, vitamine research has made great strides; nevertheless, many of the findings obtained at that time, with few exceptions, still endure to this day. Should it appear later that the human avitaminoses are associated not only with a deficiency of the corresponding vitamines, but with other correlated factors, then the significance of vitamines for life would still retain its complete justification. That the etiology in human avitaminoses may be more complicated than appears at first glance, we have already pointed out. Should it happen that there is a deficiency of one vitamine, we may be almost certain that it is not the only fault in the diet leading to the particular disease. Along with the lack of one vitamine, there may be a sub-optimal quantity of other vitamines; there may also be a protein and salt deficiency. In addition to this, it is possible that the quantitative relationship of the individual constituents may not be correct; it may likewise be (and this is very often the case in practice) that the vitamine is indeed present in the diet but not in quite sufficient quantities. Related as it is to all these factors, we must not wonder that the picture of an avitaminosis often seems to us to be clouded. Taking rickets as a concrete example, we see that although this disease is not of rare occurrence among well-to-do people, it nevertheless occurs most frequently in the poorer stratas of society, where poor living conditions and hereditary factors, together with dietary causes, may play a part. It is also not likely that the lack of vitamine A only is the cause of rickets, as is now thought to be the case; it may be associated with a partial deficiency of other factors, which chiefly consist in that the balance

between the proteins and the carbohydrates is disturbed in favor of the latter. All these conditions may affect the clinical picture of rickets as we understand it.

Most of the pathological conditions which we designate as human deficiency diseases find their replica in animal affections already described. The diseases have been produced experimentally and are regarded by our greatest specialists as identical with human avitaminoses. However, this does not exhaust our data on the subject.

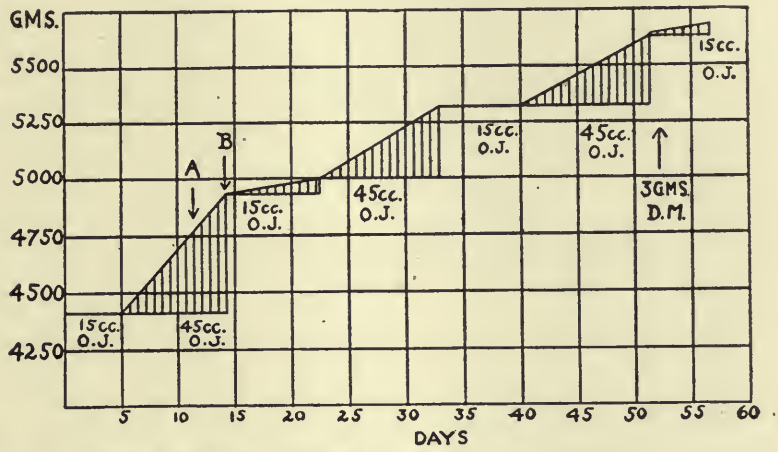


FIG. 37. THREE SEPARATE ORANGE JUICE (O.J.) ADDITIONS IN A PERIOD OF 60 DAYS GAVE UNIFORM WEIGHT INCREASES

During period I, an increase in the food produced no corresponding increase in weight. When the usual quantity of orange juice (15 cc.) was given at *B*, a second increase in food was made. The effect of the two food increases (dextri-maltose D.M.) was distinctly less than that produced by the larger quantity of orange juice. Orange juice from which the B-vitamine was removed by kaolin showed no such effect (Byfield, Daniels and Loughlin).

Exact experiments have also been conducted on man, having the convincing value of a well-planned animal experiment. Thus, we are familiar with clinical cases in which the food intake was controlled, so that the course of the particular disease could be followed quite accurately. From what has been said, it is clear that we are no longer concerned with conjectures in the classification of human avitaminoses. We are in possession of proof, which makes it certain that man needs at least three vitamines—antiberiberi (B), antirachitic (A), and antiscorbutic (C).

Especially from children's clinics, do we have observations that are in accord with the results of animal experiments. In this regard, we wish particularly to mention the work of Hess (461), who studied the influence of vitamine C on the growth and weight of infants. To be sure, Byfield, Daniels and Loughlin (l.c. 90) believed that his results must have been due to the simultaneous presence of vitamine

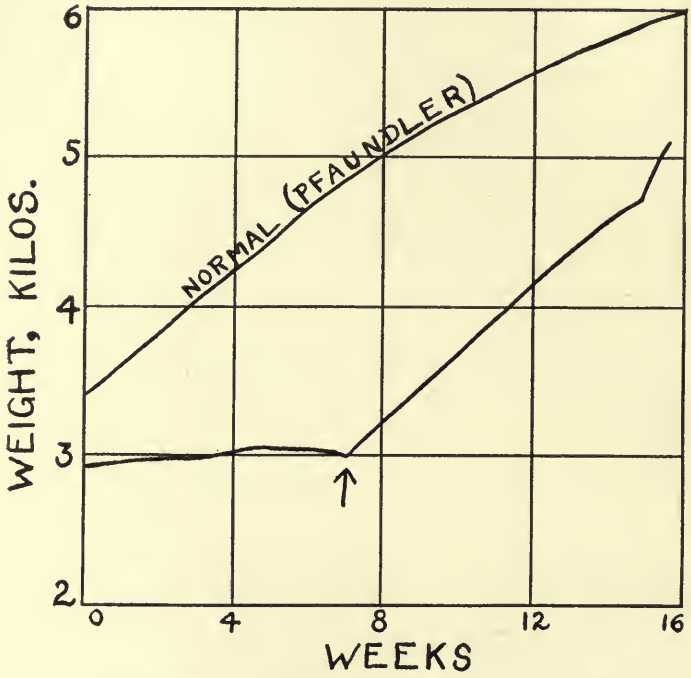


FIG. 38. BREAST-FED CHILD SHOWING STATIONARY WEIGHT

Mother received an addition of 50 grams butter and 30 grams turnip juice (vitamines A and B), at the point indicated (Dalyell; courtesy of Brit. Med. J.)

B, but the value of the experiment of Hess is not lost for our purpose. Byfield, Daniels and Loughlin were able to show that when children are given orange juice shaken with fuller's earth, thereby removing vitamine B, there is no influence on growth, whereas the untreated orange juice has a far greater effect than an addition of extra calories, in the form of an increased basal diet. This is evident from the illustration (Fig. 37). It is interesting to note that this

experiment with children was accompanied by a similar experiment with rats, with identical results. A second example of this kind is seen in the work of Chick and Dalyell (462), who studied the effect of vitamines particularly on under-nourished children in Vienna. Here, too, the growth-promoting influence of vitamine C may

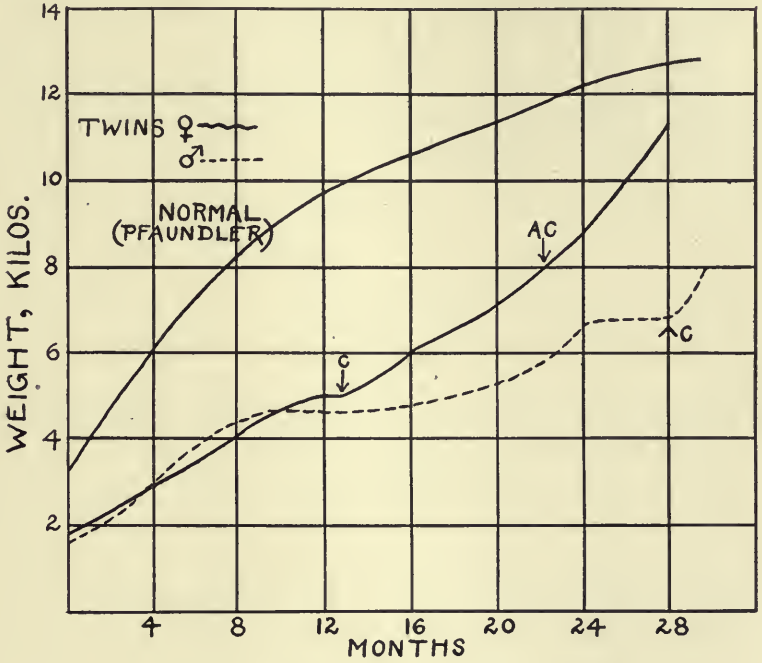


FIG. 39. TWINS, BOY AND GIRL, WITH SCURVY

The girl received at the point *C* an antiscorbutic in the form of raw milk; later at *AC*, lemon or turnip juice, with butter or cod liver oil were given. The boy was suffering from subacute scurvy and did not get any antiscorbutic except at the place marked *C*, when his growth markedly improved; he suffered also from rickets. (Dalyell; courtesy of Brit. Med. J.)

perhaps be explained by the apparent presence therein of vitamine B. The administration of vitamine to the nursing mother, with effect on the growth of the child, can find its parallel only in the most exact animal experiment.

Logically, we should begin here with the description of human avitaminoses, like beriberi, scurvy, rickets, osteomalacia, and some

which are not yet classified as such, like pellagra and war edema. Other conditions also, like ophthalmia, dental caries, and some infectious and constitutional conditions, the progress of which seems to be influenced by vitamine additions, should be mentioned here. The application to human pathology is obviously the objective point of the entire vitamine problem. We believe, therefore, that after the demonstration of the chemistry and physiology of the vitamines, as well as the vitamine content of the various foodstuffs, and the influence of the treatment these foodstuffs undergo before consumption, the reader will be better able to perceive the real significance of the vitamines in human pathology.

PART II

Chemistry, Physiology and Pharmacology of the Vitamines

CHAPTER I

The Antiberiberi Vitamine—Vitamine B

In the foregoing chapters, we have discussed facts which permitted of the assumption of the existence of at least three different vitamine types. A great number of investigations of a chemical nature have already made their appearance, dealing with the characteristics of these important substances; it may indeed be said that no single division of physiological chemistry has latterly been productive of more publications than that of the vitamines. The reason is that new facts and observations are obtained with an ease that is truly astounding. Because of this, many investigators have failed to penetrate more deeply into this subject. They have satisfied themselves, as a rule, with results obtained by indirect conclusions. This condition of marking time and useless quibbling provides an excellent opportunity for the representatives of destructive criticism which, here more than in any other field of research, is effective in hindering the solution of our problem. In spite of these difficulties, the knowledge of the nature of the vitamines progresses gradually, and we may hope that with the resumption of scientific research, after the war, the desired goal—the chemical isolation of the vitamines—is not far distant.

A question which we must touch upon is the comparison often made between the vitamines and the ferments, a relationship specially emphasized by Seidell (462a). Since the nature of the ferments, in spite of the many years of study, still remains to be cleared up, this comparison raises a poor prognosis as to the outcome of our vitamine investigations. However, we find many far reaching differences between these two classes of substances. Whereas we may observe in ferments that in a temperature interval between 50° and 70° C., there is a sudden snapping, like the over-wound main spring in a clock, we note in vitamines that the inactivation proceeds gradually with an increase in temperature. In general, the complete destruction of vitamines is seen after a much higher temperature than in ferments. The vitamines appear to us, as compared with ferments, to have a relatively simple structure, and it would be highly desirable to have more pure organic chemists take up this problem.

We come now to the discussion of the difficulties involved in the field of vitamine fractionation. Starting from any crude material and subjecting it to a variety of chemical and physical manipulations, we usually find that the vitamines are quite resistant. If now a chemical fractionation is undertaken, we note that some procedures cause either a partial or total disappearance of vitamine activity. What then is there in the natural combination which protects the vitamines against destruction? It has long been thought that it was an acid which served to protect these substances, but this is apparently only partially the case. A second possibility is that the natural combination is protective against oxidation; this may be tested experimentally and will be spoken of later.

The main difficulty of isolation, in our opinion, is that in most of the starting material used till now, the vitamines are associated with large amounts of inactive substances. When we mix a small quantity of a known substance with a large quantity of another known subtance, we find it hard to effect a separation. Here, too, we are dealing with the law of mass action, and only those substances which occur in large amounts in the mixture may be easily separated. Now, if we recall that we are dealing with cellular constituents, the chemical nature of which is still insufficiently known, then it is clear that our problem becomes much more complicated. Just how little the constituents of the cells are known becomes more evident when we attempt a fractionation of the vitamines; we immediately encounter cell components totally unknown to us. It is sought to avoid these difficulties by the choice of starting material which, in addition to a definite vitamine content, has less inactive substances. Even this procedure is uncertain, since upon concentration of the vitamines, there is a corresponding concentration of the impurities. There is no method whereby we may, in one operation, remove the vitamines from the bulk of its associated impurities, and which would be applicable to a large quantity of crude material. Besides, it is not impossible that the delay attending the usul chemical procedures may have some destructive influence on the vitamines. For example, if it should be necessary to exclude air, it is not very difficult to provide for this for a few days, but it is another matter when the procedures employed involve weeks instead of days. It is necessary, therefore, that we should try to develop some color reaction or pharmacological test for the demonstration of the vitamines, which

may provide an answer for the question as to which fraction contains the vitamine. It sometimes takes weeks before we are certain of this, and during this time the fractions obtained must await further manipulations. The means used till now for the study of the nature of the vitamines (almost exclusively the more stable vitamine B) are as follows:

1. Fractionation of the active material according to the known methods.

2. Attempts to separate the active material by solubility and selective adsorption.

3. Testing of vitamine-like action of combinations of known or unknown composition.

4. Synthetic experiments with substances obtained in vitamine work.

Up to the present, none of these four procedures has yielded very substantial results, although the first of these methods, which is most direct, has been productive of the most instructive findings. By this means, some precipitation reactions have been found which lead to a vitamine fraction which appears to be quite simple in comparison with the complexity of the starting material. From these fractions, there have been isolated well defined substances which possess a very marked curative action while in the impure state, but which, upon further purification, lose much if not all of their activity. *Nevertheless, it has been possible to isolate a substance that has been crystallized to constant melting point, at the same time retaining a good deal of its activity;* this was the case with our work on yeast. However, the objection is justified that the vitamine is present in such a small quantity that it has no effect on the melting point. We must determine now whether we are dealing with inactive material that has adsorbed the vitamine, or with one or more cleavage products serving as the inactive basic structure for the vitamines. It is obviously very unlikely that the vitamines should disappear without leaving a trace. When radium, for example, undergoes complete decomposition, the end product is inactive lead. It is not impossible that in vitamines we encounter a similar problem. Our view is further strengthened in this respect when amongst the substances usually accompanying vitamine B, we constantly meet with products, having the pyridine ring, which has been recognized as a cell constituent for the first time in vitamine work. If we were certain

that these pyridine derivatives are indicative of decomposition products of vitamines, then it would be possible to demonstrate their formula and chemical constitution with comparative ease; subsequently the synthesis could be made. Although the amount of these pyridine derivatives is small enough to permit of a possible relationship with the vitamines, our facts are not sufficiently certain to proceed to such work, since in the end we may be dealing with incidental impurities.

The second method of isolation, by extraction with various solvents or treatment with adsorptive agents, has given some interesting results, though it could finally be shown that such fractions still contained complexes. Hence a further fractionation would again be necessary, whereby the above mentioned difficulties would again have to be dealt with.

The third way, naturally only adapted to exclude from our consideration substances of known composition, did not prove of value in the studies of the nature of vitamines. Obviously, a positive result might have been due to a contamination with vitamine, or to a secondary effect that might be able to simulate a vitamine action.

The fourth, or synthetic, procedure has yielded no results till now, since the investigations have been based on weak foundations. What then is the best method for the chemical study of the vitamines? First of all, we must stabilize the vitamines, steps towards which have already been taken. If this should prove to be impracticable, we must look for another vitamine in Nature which is more stable. We shall see directly that in general, vitamine B is more stable than the two other vitamines. It would suffice if, after the chemical purification of this substance, there remained a single function which would characterize the substance as a vitamine. We have already learned that all three vitamine types are present in seeds, or that they may eventually arise through chemical re-arrangement. Although we have no positive proof that the three vitamine types are related chemically, it is not impossible that the identification of a single vitamine may be of immeasurable help in the study of the other vitamine types.

The vitamines have this in common, that they are all necessary to complete a synthetic diet. Further, none of the vitamines can be identified with any of the dietary constituents already known; they are active in very small quantities and none of them possess any too

great stability. It is possible, on the one hand, that we may need new chemical methods in order to discover their chemical nature; on the other hand, it is not unlikely that our older methods, which have been of such use to us in the identification and isolation of so many naturally occurring products, may lead us to the desired goal.

We have already pointed out the status of this question, up to 1911, in the historical part. We stated that till then, the investigators in this field were in doubt as to whether the factor curing beriberi was really a chemical substance. We have shown, together with Cooper (l.c. 68) that when pressed yeast is hydrolyzed by boiling vigorously with 20 per cent sulphuric acid for 24 hours, a ıd then the sulphuric acid completely removed with baryta, the evaporated filtrate still exhibited a very marked curative action. This stability in the presence of acid makes it appear that the substance in question is an organic base, which would distinguish itself by the known chemical characteristics of this class of substances. Based upon this assumption, we (463) undertook a systematic investigation of rice polishings.

CHEMICAL INVESTIGATION OF RICE POLISHINGS

Since no chemical reaction was known for the demonstration of this vitamine, every one of the investigated fractions was tested on beriberi pigeons. The stage at which vitamine was administered (mostly given per os in the beginning) was the appearance of contractions of the neck, wings and legs. Left to themselves, the pigeons died in about 12 hours from the onset of the above symptoms.

A series of preliminary experiments were made in order to secure an active solution of the simplest possible composition. This was obtained by shaking rice polishings, which consist chiefly of cellulose, phytin and fat, with alcohol saturated up to a certain point with gaseous hydrochloric acid. This procedure possessed the advantage that the solution was completely freed of alcohol insoluble material. The alcoholic solution was then concentrated in vacuum, yielding a fatty residue. This residue was melted on the water bath, extracted with hot water, and the layers separated in a separatory funnel while still hot. The watery extract, which was very active, was treated with sulphuric acid till a 5 per cent solution was obtained, and then completely precipitated with a 50 per cent phosphotungstic acid solution. The precipitate was decomposed with baryta and the

resulting filtrate, after removal of the excess baryta, was tested for its curative power on beriberi pigeons. The solution was very active and was free of phosphorus, protein, and carbohydrates (l.c. 463). The first difficulty encountered was that of determining the exact dose for a sick pigeon. As will be shown later, the solution contained much free choline which is very toxic for pigeons. This difficulty could be overcome by calculating the dosage on the basis of the quantity of rice polishings used. With every additional fraction, the naturally occurring loss must be made up by an increased dosage.

The fact that the solution was phosphorus-free served as a final blow to Schaumann's hypothesis regarding the lack of phosphorus, since it was demonstrated for the first time *that a solution completely free from phosphorus, was active.* Since the phosphotungstic acid filtrate was absolutely inactive, all of the active substance must have been contained in the precipitate.

The usual methods were used in the working up of such precipitates. The resultant solution, which contained a large amount of potassium and hence reacted strongly alkaline, was neutralized with hydrochloric acid and then concentrated to a syrup in vacuum. The residue was taken up in alcohol, leaving the greatest part of the potassium chloride behind. The alcoholic solution was precipitated by sublimate, the precipitate being recrystallized from water, with the addition of some sublimate. The crystals were then suspended in water and decomposed with hydrogen sulfide. This solution, as well as that obtained after the elimination of excessive sublimate, was active (the filtrate, however, being more so). Hence it was not possible to effect a separation with sublimate. The solution obtained from the precipitate was concentrated in vacuum, the residue dissolved in alcohol and precipitated with an alcoholic solution of platinic chloride. A series of fractions were obtained which, on analysis, proved to be pure choline. The alcoholic solution was still active after complete elimination of choline. It could not be doubted therefore that the curative substance did not belong to the choline group. In the mother liquor remaining after precipitation with sublimate, various precipitants were tried in order to precipitate the active substance.

For this purpose, silver nitrate in alkaline solution was used by the author (corresponding to the precipitant for the histidine group). The solution was freed from chlorine by silver sulfate. After the

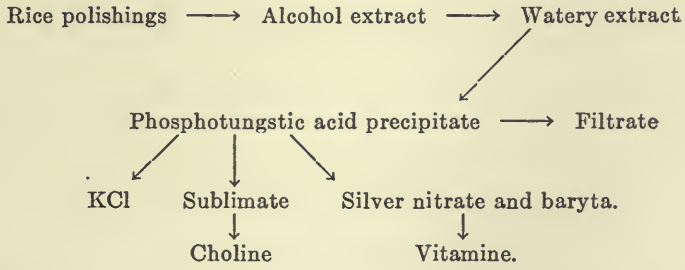
removal of silver and sulphuric acid, the solution was acidified with nitric acid, and then treated with silver nitrate till it gave a brown precipitate with baryta.

A saturated baryta solution was then added so long as a drop of the clear solution gave even a slight white precipitate on addition of ammoniacal silver nitrate solution. The precipitate was then washed free of nitric acid, decomposed with hydrogen sulphide, the last traces of baryta removed with very dilute sulphuric acid, and concentrated in vacuum. The solution thus obtained was very active. By means of the gradual evaporation of the solution in the desiccator, with the addition of alcohol, a colorless crystalline substance was obtained. This weighed 0.4 gram out of 50 kilos of rice polishings (secured from Malay). The very small yield prevented further work, it being possible only to make a series of reactions. The needle-like crystals, melted sharply at 233°C. (uncorrected) and were rather insoluble in cold water and cold alcohol. At that time, no other substance could be found in this fraction.

As to the therapeutic action of the product, we expressed ourselves only with great caution, but beyond all doubt there was a curative action present. The amount used for a curative dose contained only four milligrams of nitrogen. Smaller doses were not tried at the time.

In a later publication (l.c. 62), we called this active substance "beriberi vitamine," to make it evident that it contained nitrogen and was indispensible for life.

To illustrate our procedure, we append a schematic diagram.



Still later, we attempted to simplify our procedure (l.c. 324). In the first place, the rice polishings were extracted with ordinary alcohol instead of acid alcohol. The watery extract was then immediately

precipitated with silver and baryta, instead of phosphotungstic acid. We observed that this extract behaved quite differently from the previous one; it appeared to contain less potassium and no free choline. On decomposing the silver precipitate, a substance was obtained (not the vitamine fraction), which melted at 231°C. and was identified as allantoine. Schaumann (464) described the isolation of a substance containing nitrogen, which was perhaps allantoine but was not investigated either chemically or therapeutically.

We have therefore isolated from rice polishings two known nitrogenous substances, allantoine and choline, and the vitamine fraction. The method described in our first paper on rice polishings seems to be peculiarly adapted for the isolation of the vitamine fraction, although it has shown the problem to be more complicated than was apparent at first sight. We met with particular difficulty when the rice polishings were not extracted in the laboratory but in a chemical factory, and in large quantities. The investigation was begun with 380 kilos of rice polishings (465) using the method described in our earlier work on the same material. For technical reasons, however, it seemed necessary to remove the fat first. Ordinary instead of hydrochloric acid alcohol was used, and the evaporated extract was hydrolyzed for two hours with 5 per cent sulphuric acid. In other respects the procedure was the same as above; the individual fractions were not investigated but careful experiments were made with the vitamine fraction. The other substances, of no direct concern in our problem, were kept for later work. We thought at first that we had isolated two different substances by means of fractional crystallization of the vitamine fraction, although they seemed to crystallize out together, especially when the concentration was too great. When the first fraction was recrystallized, needle-like crystals were obtained which melted at 233°C. (uncorrected) and gave the following figures on combustion according to the method of Pregl:

4.796 mgm. substance yielded 10.34 mgm. CO_2 and 1.685 mgm. H_2O corresponding to 58.80 per cent C and 3.93 per cent H.

4.212 mgm. substance yielded 9.095 mgm. CO_2 and 1.465 mgm. H_2O, corresponding to 58.89 per cent C and 3.89 per cent H.

4.367 mgm. yielded (at 713 mm. 18°C.) 0.420 cc. N corresponding to 10.58 per cent N.

Only a small part of the nitrogen could be determined by Van Slyke's method. Nitrogen could not be estimated by Kjeldahl's

method. From the above figures, the following formula was calculated: $C_{26}H_{20}N_4O_9$. Still, as we shall see later, these figures are almost equally applicable for the formula of nicotinic acid, $C_6H_5O_2N$, containing 58.3 per cent C, 4.08 per cent H, and 11.34 per cent N. The substance seems to have been identical with that found in this fraction earlier (l.c. 463). While the weight of the original crude product was 2.5 grams, that of the recrystallized substance was 1.8 grams. On additional purification, the melting point was no higher. The mother liquor was further fractionated, yielding a substance which appeared to be more soluble in water than the first. After many recrystallizations, it melted at 234°C. (uncorrected). When treated with a watery solution of picric acid, this substance yielded a somewhat insoluble picrate, which could be recrystallized from water, melted at 218°C. (uncorrected), was light yellow in color and crystallized in prisms and plates. The following results were obtained on analysis:

4.217 mgm. yielded 9.625 mgm. CO_2 and 1.48 mgm. H_2O; 58.37 per cent C, 3.93 per cent H.
4.276 mgm. yielded 9.16 mgm. CO_2 and 1.55 mgm. H_2O; 58.45 per cent C, 4.06 per cent H.
3.11 mgm. yielded 0.315 cc. N (714 mm. 19.5°C.); 11.11 per cent N.
3.608 mgm. yielded 0.362 cc. N (712 mm. 19.5°C.); 10.97 per cent N.

For the formula $C_6H_5O_2N$ (123.05) there is 58.3 per cent C, 4.08 per cent H, and 11.34 per cent N. The picrate had the following composition:

4.471 mgm. yielded 6.74 mgm. CO_2 and 0.895 mgm. H_2O, 41.12 per cent C; 2.24 per cent H.
2.404 mgm. yielded 0.349 cc. N (707 mm. 19°C.); 15.79 per cent N.

These figures correspond to the formula $C_{12}H_8O_9N_4$ (352.12), which contains 40.9 per cent C, 2.29 per cent H, and 15.91 per cent N. The composition of the substance, as well as its picrate, characterizes it as nicotinic acid (m-pyridine carbonic acid). This substance was first found in Nature by Suzuki, Shimamura and Odake (466), and was later described in detail by Suzuki and Matsunaga (467). We shall see later that the same substance was found by us in the vitamine fraction of yeast.

From the mother liquor of the nicotinic acid, another substance was isolated which gave the Millon reaction in the crude state.

Because of the small yield, it could not be investigated further. After the publication of our findings, Schaumann (468) undertook some experiments with rice polishings, using our method. He obtained a small amount of a crystalline substance from the sublimate fraction, and demonstrated that it was active. He was able to confirm our work, but he could not effect a complete isolation. Later, a great number of investigators tried to verify our findings and succeeded, at least as far as the vitamine fraction was concerned. Among these, we wish to mention Wellman, Eustis and Scott (469), as well as Eustis and Scott (470). Vedder and R. R. Williams (471) obtained, with our method, a crystalline base that was therapeutically active. They found that unhydrolyzed extracts acted more slowly than the hydrolyzed. Besides this, they observed that the vitamine was not completely soluble in 95 per cent alcohol, and was destroyed by strong alkali; it appeared also that it could not be precipitated by basic lead acetate. The same results were obtained by R. R. Williams and Saleeby (472), substantiating the silver nitrate-baryta method. Later, Williams (473) described various modifications of our method. Issoglio (474) also found that phosphotungstic acid precipitates the vitamine. Subsequently, the isolation of vitamine from rice polishings was attempted by Kondo and Gomi (475) and by Murai (476).[1] Brill (479) tried to concentrate the vitamine by adsorption with infusorial earth, but this procedure was not very suitable. Fraser and Stanton (480) also conducted some experiments, attempting to extract and isolate the vitamine.

A much more interesting paper was published in 1912 by Suzuki, Shimamura and Odake (l.c. 466). They extracted fat-free rice

[1] Some patents have been taken out on the isolation of vitamines from rice polishings. That of Tsuzuki (477) was nothing more than a concentrated alcoholic extract of rice polishings. In addition, there is the patent of Gams and Schreiber (478) and the identical patent of the Society for Industrial Chemistry at Basle, which briefly is as follows: the purification of the extract of rice polishings, which is supposed to contain very little inactive material, consisted of a precipitation with lead acetate, first in acid and then in neutral solution. Then the filtrate was precipitated by alkaloidal reagents, like phosphotungstic acid or oxalic acid, and the resulting precipitate decomposed in a watery solution with calcium carbonate. By this means, a syrup was obtained which was soluble in methyl but not in absolute ethyl alcohol, ether and acetone; it gave a strong diazo reaction. The product is sold under the name of "Orypan."

polishings with alcohol in a reflux condenser for three hours, using fresh solvent, till the extraction was complete. The alcoholic extracts were combined and concentrated. The residue was diluted with water, sulphuric acid added (till a 3 per cent solution resulted) and precipitated with 30 per cent phosphotungstic acid. The solution obtained by decomposing the phosphotungstic acid precipitate was very active and was called "crude oryzanin I." The yield was 1.2 grams from 300 grams of fat-free rice polishings. This fraction cured pigeons promptly in amounts of 3 to 4 centigrams, while the filtrate of the phosphotungstic acid precipitate was entirely inactive. The active fraction was soluble in water and alcohol, was strongly acid and gave a marked Millon and diazo reaction. On the addition of lead acetate, a slight precipitate was obtained, increasing on the addition of ammonia. The substance was partially precipitated by sublimate, mercuric acetate, and mercuric nitrate. An attempt to separate this active fraction resulted in a complete loss of activity. This was never the case in our experiments; the vitamine was very resistant to acids.

The hydrolysis was accomplished by heating for two hours with 3 per cent hydrochloric acid. On cooling, yellowish brown crystals separated out, which were recrystallized from hot alcohol. In this way two substances were obtained, the first of which was less soluble than the other. Both were difficultly soluble in water and gave an acid reaction; they were soluble in alkali, and could be reprecipitated on the addition of acid. On analysis, the first product gave the formula $C_{18}H_{16}N_2O_9$ and was called α-acid; the second product gave the formula $C_{10}H_8NO_4$ and was called β-acid; both showed the Millon and diazo reactions. Unfortunately, the melting point and the investigation of the derivatives was not recorded in the report of the Japanese investigators. From the hydrolyzate, choline and nicotinic acid (m.p. 214°C.) could be isolated as picrates; glucose was also found. One hundred parts of crude oryzanin gave 10 parts of α- and β-acids, 30 parts of choline and nicotinic acid, and 23 parts of glucose. One gram of crude oryzanin yielded 0.044 gram nitrogen, of which 0.035 gram could be precipitated by phosphotungstic acid, of 0.009 gram was in the form of α- and β-acids.

As this composition shows, the investigation of rice polishings by the Japanese workers yielded fundamentally different results from ours. The crude oryzanin I. was then subjected to further

purification; it was dissolved in water and precipitated with a 20 per cent tannin solution till only a slight cloudiness was seen. The precipitate was filtered off and quickly washed with a 1 per cent tannin solution. It was then rubbed up in a mortar with 3 per cent sulphuric acid till a complete solution resulted. This was treated with an excess of baryta, the precipitate filtered off, and the excess of baryta removed from the filtrate with dilute sulphuric acid. The solution was shaken out with ether and concentrated. In this way, a preparation was obtained, called crude oryzanin II., which was three times as active as the first preparation.

When a concentrated watery solution of this preparation was rubbed up with a slight excess of dilute picric acid, a flocculent precipitate settled out which became crystalline on standing in the cold. These crystals still occluded some nicotinic acid picrate, but with careful technique the latter remained in solution. The oryzanin picrate was recrystallized by dissolving in cold acetone and allowing this to evaporate in the dessicator; yellowish brown microscopic needles, grouped in star formation, were obtained. An amount corresponding to two centigrams of picrate was very active for pigeons; the substance was given only to two pigeons. The amount of picrate obtained was so slight that there was not enough for a melting point. The question as to whether the pure oryzanin would give the same decomposition products as oryzanin I. was therefore left open by the authors. Since the publication of this work in 1912, nothing of a corroborative nature has been printed by the Japanese investigators (at least to our knowledge). In the meantime, Drummond and Funk (481) tried to confirm the above findings, but all attempts to isolate the substance as a picrate failed. Above all, it was evident that an extract of rice polishings is still quite a complicated mixture. Of the many substances it contains, we were able to isolate, besides the previously mentioned choline, allantoine and nicotinic acid, also betaine, adenine, guanine and apparently guanidine. An observation, made accidently, showed us how careful one must be in drawing conclusions from such fractionations. In this instance, an apparently pure substance was isolated, which had a constant melting point on recrystallization. The substance consisted of betaine and nicotinic acid, which could not be separated from each other by recrystallization. Only when the nicotinic acid was separated as a copper salt, was the betaine apparent. It was also

clear from this work, that Barger (482) was correct in his statement that the substance described by us in 1913, to which we then gave the formula $C_{26}H_{20}N_4O_9$, was in reality nicotinic acid. However, the view expressed in the Report of the Medical Research Committee (l.c. 333), that the substance isolated was nicotinic acid contaminated with vitamine is erroneous, since the analysis indicated, at the time, pure nicotinic acid for which no curative action was claimed. Summarizing our work with rice polishings, we were able to differentiate the curative substance of 1911, but only when we undertook the extraction of the rice polishings ourselves. When this was done in the factory no curative substance was obtained, and hence no publication was made, thus explaining the non-appearance of the protocols of the animal experiments. Therefore, it is not justifiable to apply the conclusions drawn from our negative results with rice polishings to our positive yeast findings, which we shall describe in the next chapter.

In conclusion we wish to call attention to an investigation by Hofmeister and Tanaka (483). The impression was given that the active vitamine had been isolated in the pure state from rice polishings. It will be well to describe Hofmeister's method, different from any previously used. He began his work with the notion that our vitamine is, in reality, nicotinic acid, but this is not the case, since in every case where nicotinic acid was isolated there was no curative action.

Hofmeister shook rice meal three times with double the volume of 80 per cent alcohol on the shaking machine. The filtered solution was evaporated in the presence of a stream of air, in vacuum. Then the residue was acidified up to 3 per cent with hydrochloric acid, the fatty acids extracted with ether, the ether removed, the solution concentrated to a syrup in vacuum at a low temperature, and again taken up with 80 per cent alcohol to free it from colloidal impurities. The clear filtrate was made faintly alkaline with sodium carbonate, taking care to prevent an excess of alkali, and precipitated by bismuth potassium iodide (prepared according to Kraut) with constant stirring. It is necessary here to avoid a strongly acid reaction, else the active substance precipitates too. The dirty grayish-yellow precipitate of the choline fraction was filtered after standing for 5 hours; to the filtrate, was added one-tenth of its volume of 20 per cent hydrochloric acid, and the active substance precipitated out

with the same reagent. The powdery, brick to scarlet red precipitate is quickly filtered off by suction, thoroughly drained, carefully rubbed up in a mortar with silver carbonate, and immediately filtered. The weakly alkaline solution, containing silver, is acidified at once with hydrochloric acid, filtered free from silver chloride and evaporated in vacuum almost to dryness. The residue crystallizes in weakly colored, radiating deliquescent crystals. From the crude hydrochloride, of which 5 to 10 mgm. was sufficient to cure a pigeon in 24 hours, and which retained its activity for 8 to 10 days, a yellow precipitate was obtained with gold chloride, which was amorphous at first and then crystalline, and which crystallized from water in plates and flat prisms. The quantity of aurate obtained corresponded evidently to the amount of hydrochloride used (m. p. 273.5°C., which on further recrystallization rose to 277° C., uncorrected). This aurate was not yet entirely pure and was, therefore, converted back to the hydrochloride and recrystallized from water; from the purer crystals, a preparation was obtained, poor in ash, which melted at 240°C. and gave the formula $C_5H_{11}NO_2$. HCl. From this preparation, the pure chlor-aurate was then prepared and analyzed. The free base, called "Oridin," is a white, powdery, hygroscopic substance, easily soluble in water with a slight acid reaction; it is slightly soluble in cold absolute alcohol but quite so in hot. From the latter, the base may be obtained in crystals with ether. When these are heated, pyridine-like vapors are given off. In water, precipitates were obtained with phosphotungstic and phosphomolybdic acid, bismuth potassium iodide and gold chloride, but not with bromine water, platinic chloride or sublimate. The phosphotungstic acid precipitate is soluble in acetone, but not very soluble in water. The watery solution does not dissolve any copper carbonate and gives no color reaction with iron salts. Isonitrile and mustard oil reactions were negative. On boiling with hydriodic acid and phosphorus, alkaline vapors are evolved, but not with sodium hydroxide. With calcium hydrate a pyridine odor is obtained. The purest preparation gave only a very faint pine splinter reaction. No color was obtained with formaldehyde and sulphuric acid, or on heating with nitric acid and on addition of alkali. The formula is isomeric with betaine, and the substance is perhaps a dioxypiperidine. The pure substance was inactive, and Hofmeister seemed uncertain as to whether the active substance had been destroyed or had passed into the filtrate. We

have often tried to precipitate the vitamine with gold chloride but without succeeding. Besides, as we have lately again noted, a number of substances including nicotinic acid, are precipitated by bismuth potassium iodide. According to Hofmeister (l.c. 483) colamine, proline, hematine, papaverine, trigonelline and stachydrine have no influence on beriberi in pigeons. We have tried the above procedure with tomatoes but found it impractical.

CHEMICAL INVESTIGATION OF YEAST

The chemical aspect of vitamine B has been neglected in most of the books and reviews on the subject. We propose to describe only the indisputable facts in order to give the reader the opportunity of forming his own opinion of the status of the question. It is too soon to say whether the isolation of this vitamine has been successful or not. In any case, the investigation on yeast extract made by us is one of the few researches upon which we may build further. We have investigated yeast in various ways, and yet we do not know if the vitamine occurs free or combined. The facts uncovered up to the present point to both possibilities.

In our first attempt to isolate the vitamine from yeast (l.c. 324), 75 kilos of dried yeast were extracted with alcohol, the extract concentrated in vacuum and the residue hydrolyzed with 5 per cent sulphuric acid for five hours; after cooling, it was filtered from separated fatty acids, the filtrate diluted with an equal volume of water and precipitated with phosphotungstic acid. The precipitate was decomposed in the usual manner, and the resulting filtrate concentrated. A concentrated watery solution of silver nitrate was then added till a drop of the clear solution gave, with a saturated baryta solution, instead of a white precipitate, a brown precipitate of silver oxide. The solution, freed from the heavy precipitate of purine bases, was treated with saturated baryta so long as it gave a white precipitate with ammoniacal silver nitrate solution. The silver precipitate was decomposed with hydrogen sulphide and the filtrate, after the removal of the last traces of baryta, concentrated to small volume in vaccum; the residue was transferred to a dish with a little alcohol and allowed to stand in the dessicator, whereupon crystals separated out weighing 0.6 gram. They were recrystallized from dilute alcohol yielding 0.45 gram of colorless needles (see fig. 40)

melting at 233° C. This substance from yeast was therapeutically active; 2 to 4 centigrams were sufficient to cure a sick pigeon in a short time; smaller doses were not tried. The substance could be precipitated by mercuric acetate but not by the nitrate or sulphate. It gave no precipitate with a nitron solution—showing the absence of nitric acid—and had a neutral reaction. No blue copper salt was obtained with copper oxide, which would indicate that the substance is not an amino-acid. The filtrate from the silver precipitate was inactive.

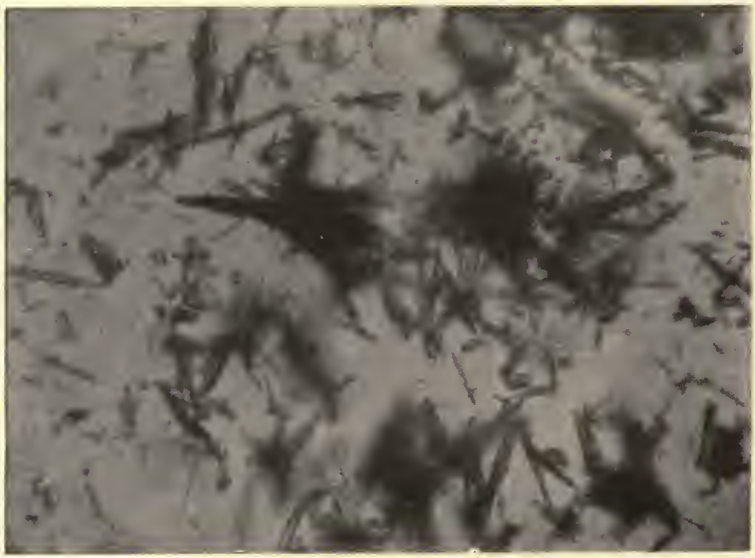


FIG. 40. MICROPHOTOGRAPH OF CRYSTALS OBTAINED FROM VITAMINE FRACTION OF YEAST, POSSESSING CURATIVE ACTION

Another batch of yeast was treated in a somewhat different manner. The alcoholic extract, after concentration in vacuum, was simply taken up with water, and not hydrolyzed as above. Thus the vitamine was obtained in watery solution. This was then precipitated with phosphotungstic acid and treated as above; in this case however, the silver fraction did not yield an active product, a substance being isolated which could be identified as uracil. All the vitamine, in this case, was found in the filtrate from the silver nitrate precipitate, which might mean that the vitamine of yeast occurs

chiefly in combination. This filtrate was then hydrolyzed with sulphuric acid, and the subsequent procedure again repeated. Here too we met with a mixture of pyrimidine bases, among which thymine could be demonstrated, so that finally after a long procedure, only a

FIG. 41. PIGEON BEFORE TREATMENT

FIG. 42. THE SAME PIGEON (CF. FIG. 41) CURED WITH 8 MGM. OF YEAST VITAMINE AFTER TWO HOURS

trace of active substance was found. This observation shows that in working with vitamines, the number of manipulations had best be kept down to a minimum, if results are to be obtained.

Later on (485, and l.c. 465), we again investigated the vitamine fraction, using 2.5 grams of the crude fraction, obtained from 100

kilos of dried yeast, according to the method described. The substance was obtained from the decomposition of the silver baryta fraction, and possessed a crystalline structure, melting at 210° C. (uncorrected). This crude vitamine fraction was a splendid thera-

FIG. 43. PIGEON BEFORE TREATMENT

FIG. 44. SAME PIGEON (CF. FIG. 43) AFTER THREE HOURS, WITH 4 MGM. OF YEAST VITAMINE

peutic agent in a great number of beriberi pigeons. As the accompanying photograph shows (fig. 41-46), the preparation cured pigeons completely in a very short time (2 to 3 hours). Further data on this subject will be found in the protocol appearing at the end of this chapter, showing, in the form of a table (p. 185), the action of substances

obtained from yeast. The dose of crude fraction used was 4 to 8 mgm., and the preparation was administered intramuscularly. The product gave a strong uric acid and phenol reaction with the Folin-Macallum reagents (486).

FIG. 45. PIGEON BEFORE TREATMENT

FIG. 46. SAME PIGEON (CF. FIG. 45) AFTER THREE HOURS, WITH 8 MGM. YEAST VITAMINE

This 2.5 grams of crude fraction was then recrystallized from dilute alcohol, and gave, at first, 1.6 grams of a substance crystallizing in microscopic needles. Even after several recrystallizations, the melting point could not be raised above 229° C. (corrected). The first crystallization still gave the reactions mentioned above; only

when the substance was recrystallized twice did these reactions disappear. The purified substance was compared with that obtained before, and mixed melting points were taken, whereby the new product melted four degrees lower. Mixed in equal quantities, both substances melted at 229°C. (corrected). They were absolutely identical as regards solubility, reactions and crystalline form. The substance gave a white precipitate with Millon's reagent, but the color reactions were always negative. In a 0.74 per cent solution, a definite rotation in the polarimeter could not be shown. On drying in vacuum at 100° C. and analyzing, the following figures were obtained:

3.267 mgm. yielded 6.63 mgm. CO_2 and 1.05 mgm. H_2O; 55.35 per cent C, 3.60 per cent H.
4.224 mgm. yielded 8.425 mgm. CO_2 and 1.36 mgm. H_2O; 55.72 per cent C, 3.69 per cent H.
4.256 mgm. yielded 8.66 mgm. CO_2 and 1.36 mgm. H_2O; 55.50 per cent C, 3.58 per cent H.
3.048 mgm. yielded 0.373 cc. N. (710 mm. 15.5°C.) 13.53 per cent N.
3.627 mgm. yielded 0.430 cc. N (712 mm.; 14°C.) 13.21 per cent N.
3.286 mgm. yielded 0.401 cc. N (705 mm.; 18°C.) 13.28 per cent N.

These figures correspond to the formula $C_{24}H_{19}O_9N_5$ (521.24), which gives 55.25 per cent C, 3.68 per cent H and 13.44 per cent N. Since a definite crystalline substance is noted on the tube when drying at 100° C. in vacuum, analyses were made after the substance was dried in vacuum over sulphuric acid; the following figures were obtained:

3.733 mgm. gave 7.775 mgm. CO_2 and 1.185 mgm. H_2O; 56.80 per cent C, 3.55 per cent H.
3.538 mgm. gave 7.35 mgm. CO_2 and 1.195 mgm. H_2O; 56.66 per cent C, 3.78 per cent H.
3.174 mgm. gave 0.372 cc. N (702 mm.; 17°C.) 12.74 per cent N.

These figures correspond to the formula $C_{26}H_{21}O_9N_5$, which gives 57.01 per cent C, 31.87 per cent H and 12.81 per cent N. From this, it appears that on drying at higher temperatures a substance rich in carbon is lost. This characteristic and the great difference in elementary composition excludes the possibility that this substance was nicotinic acid. We have recently attempted to secure further evidence in this connection by the use of the yeast method. If the nitrogen in this substance is estimated by the method of

Kjeldahl, only a third of it is determined. 0.1328 gram needed 4.0 cc. $\frac{N}{10}$ H_2SO_4; found, 4.21 per cent N. If the substance is titrated with sodium hydroxide against phenolphthalein, then 0.0373 gram requires 2.8 cc. $\frac{N}{10}$ NaOH. For a four basic acid of the formula $C_{24}H_{19}O_9N_5$, the calculated amount is 2.8 cc. That this substance is of an acid nature, is very important and we shall consider this phase of the matter again. As regards the therapeutic action of substance I, examples are given in the table (p. 185).

The filtrate and mother liquor combined, of substance I, which gave a strong phenol and uric acid reaction, were precipitated by picric acid; a crystalline precipitate was obtained, amounting to 0.9 gram; which was first recrystallized from a mixture of acetone and alcohol and then from water. The once recrystallized substance still gave a positive phosphotungstic acid reaction. On purification (light yellow needles), the melting point was raised from 217° to 219° C. (uncorrected) and gave the following figures on combustion:

4.70 mgm. gave 7.115 mgm. CO_2 and 0.98 mg. H_2O; 41.29 per cent C; 2.33 per cent H.
2.966 mgm. gave 0.436 cc. N (708 mm., 19.5°C.), 15.99 per cent N.
4.212 mg. gave 0.61 cc. N (711 mm., 18°C.), 15.89 per cent N.
0.1061 grain gave 0.1652 Nitron picrate, 65.91 per cent picric acid.
0.1261 grain gave 0.1951 Nitron picrate, 65.49 per cent picric acid.

Calculated for $C_{12}H_8O_9N_4$ (352.12) — 40.9 per cent C, 2.29 per cent H, 15.91 per cent N and 65.05 per cent picric acid. This picrate was quantitatively decomposed with nitron, and on concentrating gave 0.45 gram of silky, lustrous, colorless needles, the melting point of which, after recrystallization, rose to 235° C. (uncorrected). The crystals were very easily soluble in water and gave no color reactions. When dried at 100°C. in vacuum, the analysis gave the following figures:

3.432 mgm. yielded 7.345 mgm. CO_2 and 1.10 mgm. H_2O; 58.37 per cent C; 3.62 per cent H.
3.784 mgm. yielded 8.11 mgm. CO_2 and 1.325 mgm. H_2O; 58.45 per cent C. 3.92 per cent H.
3.415 mgm. yielded 0.343 cc. N. (702 mm., 18.5°C), 10.87 per cent N.
2.562 mgm. yielded 0.252 cc. N (15.5°C.), 10.92 per cent N.

Calculated for $C_6H_5O_2N$ (123.05) — 58.3 per cent C, 41.08 per cent H and 11.34 per cent N. The substance was evidently nicotinic acid.

Mixed with the nicotinic acid from rice polishings, it showed the same melting point, and the picrates behaved similarly.

The mother liquor of the picrate precipitate, which gave a strong uric acid and phenol reaction, was shaken out in acid solution with ether in order to remove the picric acid. After elimination of the hydrochloric acid, the solution was slowly evaporated, yielding 0.4 gram of a substance melting at 210° C. and when recrystallized from dilute alcohol to constant melting point, melted at 222 to 223° C. (microscopic needles, somewhat more soluble in water than substance I). The pure substance no longer gave the uric acid nor the Millon reaction, but the phenol reaction was still positive. For analysis, one sample was dried in the vacuum dessicator over sulphuric acid at ordinary temperature; another was dried in vacuum at 100° C. and another, at 115° C. The substance dried over sulphuric acid gave the following figures:

3.559 mgm. gave 7.745 mgm. CO_2 and 1.21 mgm. H_2O; 59.35 per cent C; 3.80 per cent H.
3.445 mgm. gave 0.375 cc. N (706 mm. 17°C.), 11.90 per cent N.
Dried in vacuum at 100°C.
4.172 mgm. gave 9.065 mgm. CO_2 and 1.535 mgm. H_2O; 59.26 per cent C; 4.12 per cent H.
2.716 mgm. gave 0.295 cc. N and (711 mm. 15°C.) 12.04 per cent N.
Dried in vacuum at 115°C.
3.694 mgm. gave 7.99 mgm. CO_2 and 1.22 mgm. H_2O; 58.99 per cent C; 3.70 per cent H.

These figures correspond equally well to two formulas, though somewhat better to the second.

	CALCULATED FOR	
	$C_{23}H_{18}O_7N_4$ (462.22)	$C_{29}H_{23}O_9N_5$ (585.28)
	per cent	*per cent*
C	59.71	59.46
H	3.84	3.96
N	12.12	11.97

To recapitulate briefly the yeast fractionation, we have been able to isolate three different substances from the vitamine fraction, which gives a strong uric acid and phenol reaction.

SUBSTANCE	MELTING POINT	URIC ACID REACTION	PHENOL REACTION	REMARK
	°C.			
$C_{24}H_{19}O_9N_5$ (dried at 100°C)	229	−	−	Gives no picrate
$C_{26}H_{21}O_9N_5$ (dried at ordinary temperature).....	223	−	−	Gives no picrate
$C_{29}H_{23}O_9N_5$.....	222–223	−	+	Gives no picrate
$C_6H_5O_2N$.....	235	−	−	Gives picrate Nicotinic acid

Aside from this, the first substance, on heating in vacuum, loses a small amount of substance rich in carbon, giving lower analytical results. Although the statement is often made that we had obtained only impure nicotinic acid, it is not justified, since the analytical figures are too far apart from the composition and the characteristics of this acid. Regarding the therapeutic action of these substances, many experiments were performed, some of which naturally turned out negative. In the table, the product $C_{26}H_{21}O_9N_5$ is called substance I, and $C_{29}H_{23}O_9N_5$, substance II.

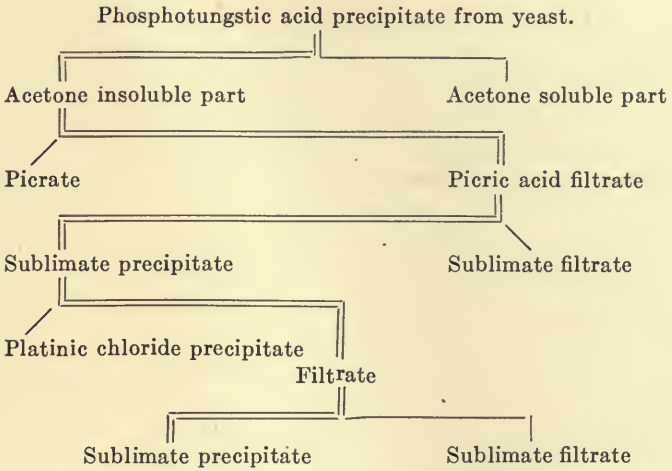
NUMBER OF ANIMALS	SUBSTANCE	DOSE	ACTION	DAYS SURVIVED
		mgm.		
1	Crude crystallization	4	Cured in 3 hours	4
2	Crude (fig. 41–42)	8	Cured in 2 hours	6
3	Crude (fig. 43–44)	4	Cured in 3 hours	4
4	Crude (fig. 45–46)	8	Cured in 3 hours	4
5	Substance I	8	Partially cured in 7 hours.	3
6	Substance I	4	Improvement	4
7	Substance I	2	Cured in a few hours	3
8	Substance I + Nicotinic acid	5+2	Cured in 2½ hours	4
9	Substance I + Nicotinic acid	4+2	Cured in 3 hours	5
10	Substance I + Nicotinic acid	3+2	Cured in 2 hours	6
11	Substance I + Nicotinic acid	4+2	Cured in 2½ hours	7
12	Substance I + Nicotinic acid	3+2	Cured in 4 hours	4
13	Nicotinic acid	10	Negative	2
14	Nicotinic acid	5	Slight	3
15	Nicotinic acid	4	Negative	2
16	Nicotinic acid	4	Negative	1
17	Substance II	5	Negative	−
18	Substance II	5	Negative	1

All these substances were administered to the pigeons intramuscularly, and from the results, it is evident that substance I had the greatest therapeutic action.

Others also investigated this question. Barsickow (487) concluded that the therapeutic action was inherent only in the living or dead yeast cells, and for the most part, he found the extracts inactive; this has, of course, been shown to be incorrect. Edie, Evans, Moore, Simpson and Webster (488) did not hydrolyze the yeast, but treated the alcoholic extract, after the concentration of the solvent, directly with silver nitrate and baryta. They obtained in this way a hygroscopic syrup which was very active for pigeons, 6 mg. being sufficient, as a rule, for a cure. On further purification, a crystalline substance was obtained which, because of the small yield, could not be purified further. This crystalline substance was analyzed but the data on its therapeutic action were lacking.

Another method, useful for the purpose of removing a large mass of inactive material, was described by us (489) in 1916. This method is based upon the separation of the phosphotungstates by means of the varying solubility in acetone. The residue, obtained from the alcoholic extract of 100 kilos of dried yeast, was extracted with 10 per cent sulphuric acid, this extract was then diluted half again with water and precipitated with phosphotungstic acid. The resulting precipitate, weighing 2237 grams was triturated several times with dry acetone, leaving in the end 144 grams of insoluble material. This portion, which represents 5.1 per cent of the total precipitate, contained all the vitamine, according to our animal experiments. The precipitate was decomposed with neutral lead acetate[2] and the resulting solution precipitated with picric acid. The voluminous precipitate consisting of adenine picrate was filtered off. It was impossible to crystallize the vitamine from the mother liquor, making it necessary to continue the fractionation with sublimate, platinic chloride, and picrolonic acid. The course of the fractionation, which was controlled by animal experiments, is shown in the diagram below, in which the double lines indicate the fraction containing the vitamine. According to a patent by Böhringer and Sons (491) the

[2] We also attempted to decompose the precipitate by shaking it out with amyl alcohol in acid solution according to Van Slyke (490), but the method was not quantitative because the last traces of phosphotungstic acid are removable only with great difficulty.



phosphotungstic acid in the above method was substituted by phosphotitanic acid. Cooper (492) then showed that when pressed yeast is allowed to stand for 35 hours at 35° C., the mass becomes semi-liquid and on filtration from the cell residue, a liquid called "autolyzed yeast" is obtained, which contains almost all of the vitamine of the yeast. It was shown later that the yeast constituents are not completely autolyzed, since if the liquid is heated to 60 to 70° C., an appreciable amount of protein separates out. When the acetone method was applied to this liquid, the amount of the acetone insoluble portion was much larger—about 1125 grams from 5 kilos of pressed yeast. We then showed that when the phosphotungstates of choline, betaine, stachydrine, guanine, adenine, guanidine, creatinine and nicotinic acid were tested for their solubility in acetone-water solutions of various dilutions, the solubility was as follows:

25 per cent acetone → 100 per cent → 50 per cent → 75 per cent

Choosing these four concentrations, the solubility was least in 25 per cent acetone and greatest in 75 per cent acetone. We were then able to show, in our work with Dubin (493), that autolyzed yeast contains much more vitamine than vitamine extracts obtained from yeast by various solvents. For this reason, autolyzed yeast would be superior to all other extracts for the purpose of vitamine fraction-

ation, if a large quantity of inactive extractives did not go into solution at the same time. If the phosphotungstic acid precipitate from autolyzed yeast is treated directly with dry acetone, almost a third of it remains insoluble, including a large amount of purine derivatives. If the acetone-water method is used, as outlined above, we obtain from the above-mentioned precipitate, weighing 1125 grams, the following fractions:

ACETONE CONCENTRATION	AMOUNT SOLUBLE	
per cent	*grams*	*per cent*
25	522	46.4
100	190	16.8
50	155	13.7
75	108	9.6

Total precipitate—1125 grams.
Insoluble residue—150 grams = 13.3 per cent.

This method might be of importance but in this case, no animal experiments were carried out.[3]

A method indicative of great progress at the time was described by Seidell (495). If, for example, 1 liter of autolyzed yeast is shaken with 50 grams of Lloyd's reagent (fuller's earth), allowed to stand for several hours and the residue filtered off and washed with dilute hydrochloric acid, vitamine B is quantitatively removed from the solution, and the filtrate is completely inactive. The great hopes held out for this method, however, have not materialized. In the first place, it is very difficult to remove the vitamine from the fuller's earth, and secondly, a number of other substances, difficult to remove, are adsorbed together with the vitamine. In the end, R. R. Williams and Seidell (496) were able to extract the vitamine from the fuller's earth with 5 per cent sodium hydroxide, at the same time showing that the vitamine was contaminated with considerable adenine. Osborne and Leavenworth (496a) showed that dilute alkali has no destructive action of vitamines, especially if the contact is of short duration. For liberation of vitamine from activated fuller's earth all those methods are suitable which are used in freeing alkaloids

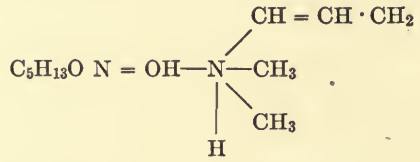
[3] In the meantime, this method was elaborated by Drummond (494) in that he investigated the solubility of various phosphotungstates, and discussed the practical applicability of these data.

from fuller's earth preparations by Rhodehamel and Stuart (496b). Recently, Seidell (496c) has combined the fuller's earth method with our silver method and has obtained some promising results. He liberates the vitamine with a baryta solution, the procedure being hastened as much as possible. From the filtrate, the purine bases are eliminated with silver nitrate while the vitamines are precipitated by ammoniacal silver nitrate. This fraction can be reprecipitated in the same way and can be analyzed for its silver content. A crystalline substance was isolated from this fraction, but it proved inactive, showing it to be a fairly complicated mixture. We found that nicotinic acid is also precipitated by ammoniacal silver nitrate. For the liberation of vitamine from activated fuller's earth, we have likewise used ammonia and pyridine; we were also able to confirm the findings of Eddy, Heft, Stevenson and Johnson (l.c. 129e) that vitamine can be adsorbed with a specially prepared charcoal from which it can be liberated with glacial acetic acid. Voegtlin and White (497) adsorbed the vitamine with mastic, and with colloidal arsenic sulfide, in which case the impurity was guanine instead of adenine.

Drummond (498) attempted to confirm our findings on vitamine B, and although he was able to do so for the most part, he concluded that our chemical work was of no particular significance, because he held that the vitamines were not precipitated by the various precipitation methods, but were adsorbed by the precipitates. The characteristic, adsorbability, is peculiar to many pure chemical substances. We need only recall Lloyd's reagent which, as we have personally proved by tests on rats, quantitatively adsorbs strychnine and other alkaloids. Pure glucose is also adsorbed in very appreciable amounts by various kinds of blood charcoals. Besides, Drummond carried out the silver nitrate-baryta precipitation in a manner different from ours, and used rats as experimental animals, which, as we shall see, behave differently than do pigeons and chickens.

Abderhalden and Schaumann (499) have recently published considerable work on yeast, which unfortunately is described so unclearly that it is hard to state whether or not progress has been made. These authors declare, in accord with the phosphorus theory of Schaumann, that vitamine B of yeast occurs as a nucleoproteid. They were able to isolate an active nucleoproteid from yeast. Pigeons receiving a half gram of this substance daily lived for 70

days. We have already seen that such findings possess no special significance, since the activity of the nucleoproteid might have been due to adsorption, as with fullers earth. The authors also assume that the mother substance of the vitamine varies according to the source of material. The vitamine was obtained from yeast extracts by precipitation with acetone, and purified by several reprecipitations. This crude fraction was investigated physiologically, and found curative for beriberi, but it could not keep pigeons alive for any prolonged period. Another preparation was obtained from yeast by treatment with alkalis. This substance did not cure beriberi, but when added together with the acetone preparation, its effect was to prolong life. A number of substances were isolated from the acetone preparation, by reprecipitating with sublimate, among which there was apparently only a single new substance obtained in the pure state. This base, called "Aschamine" had the structure of dimethylpropenylamine, the formula being



it was inactive for beriberi. In the other fractions, betaine was found as an impurity. All of these substances were prepared from hydrolyzed yeast, and the authors believed that the active substance belonged to the betaine group, having its characteristic structure. In a later experiment, Abderhalden (500) states that the active substance cannot be quantitatively removed from yeast by absolute alcohol or absolute alcohol and acetone.

Sugiura (501) placed yeast and also carrot extracts in collodion sacs, and permitted the contents to dialyze through the membrane, whereupon the product crystallized on the outside of the membrane. It was evidently hoped to prepare the active substance in the pure state by this simple procedure, but naturally the entire yeast mixture dialyzed out.

Osborne and Wakeman (502) described a method which promised greater success. The procedure consisted chiefly in eliminating the autolysis of the yeast cells by washing them and boiling with water, to which some acetic acid has been added, thus coagulating

the protein and obtaining an extract free from the products of autolysis. Concretely, 4.5 kilos of fresh yeast, corresponding to 830 grams of dry yeast, was crumbled into boiling water, the mass centrifuged, the residue washed with water and again centrifuged. Altogether, 15 liters of water were used, the combined extracts were evaporated to two liters and poured into three liters of 93 per cent alcohol, giving a 53 per cent alcoholic content by weight. The precipitate, which was inactive, was filtered off and the filtrate, together with the washings, evaporated to 300 cc. This liquid was poured into 1960 cc. of 93 per cent alcohol, giving a 79 per cent alcoholic content. The second precipitate was dissolved twice in water and precipitated with alcohol so that the alcoholic content should be 90 per cent. This fraction was supposed to contain almost the entire quantity of vitamine B present in the yeast, and amounted to 6.2 per cent of the dry yeast. The preparation had an acid reaction and gave a heavy precipitate with lead acetate and sublimate; 25 per cent of this fraction could be precipitated with silver nitrate-baryta. This work shows that the vitamine of non-hydrolyzed yeast is insoluble in strong alcohol. In our experiments this method did not prove successful, as in each fresh sample of yeast the limits of precipitation have to be established anew.

There is also the report on the chemistry of yeast by Myers and Voegtlin (503). They showed that vitamine B could be extracted from yeast by shaking the latter with olive oil; the procedure obviously possesses no particular advantage over the usual extraction, so that later they used methyl alcohol acidified with hydrochloric acid for extraction. The resulting extract was concentrated in vacuum and the residue extracted several times with dilute hydrochloric acid. In this extract, the purine bases were precipitated with silver acetate. The precipitate was filtered off and more silver acetate added to the filtrate, followed by a saturated solution of baryta. This precipitate was decomposed in the usual manner, and the histidine removed from the filtrate with mercuric sulphate. The vitamine was then precipitated from the filtrate with absolute alcohol. The precipitate was dissolved in water and freed from mercury, whereupon a very active solution was obtained. This solution gave a brown diazo reaction and contained a product similar to histamine. When the solution was concentrated, crystals were obtained which were active only so long as they contained some mother liquor. On washing the

crystals with alcohol, the crystalline form changed from spindles to prisms. If these prisms were recrystallized from water, spindles were again obtained. In this experiment, the vitamine was inactivated by drying. The adsorption methods were shown to be unspecific.

OTHER SOURCES OF SUPPLY

Hulshoff Pol (l.c. 59 and 504) reported further experiments with the X-acid of Katjang idjoe beans (Phaseolus radiatus) without giving more details on the chemistry of the substance. In 1912, we investigated milk (l.c. 324) with this point in view. As a source of supply, we used a dried milk preparation, sold under the name "Trumilk." This was extracted with alcohol and ether. The combined extracts were concentrated and the residue hydrolyzed with 10 per cent sulphuric acid for 5 hours. In this way, 51 grams of phosphotungstic acid precipitate were obtained from 1398 grams of dried milk. The silver-baryta fraction was prepared from this precipitate in the usual manner. The decomposed precipitate yielded a small amount of crystals melting at 230° C., and was curative for pigeon beriberi. In the same way, it was possible to prepare from 2180 grams dried ox brain (l.c. 324) 220 grams of phosphotungstic acid precipitate, and to obtain a trace of a crystalline substance from the silver-baryta fraction, melting at 203° C. and having curative properties. Voegtlin and Towles (505) investigated extracts of spinal cord and found that autolyzed extracts were more active than natural extracts. .

From 42 liters of commercial lime juice (l.c. 324), we obtained 1200 grams phosphotungstates, from which there resulted 5.9 grams of a vitamine fraction. Although this fraction did not crystallize out, the solution was very curative for pigeon beriberi. From our information of the vitamines in the above mentioned publication, the presence of vitamine B could indeed be demonstrated, and yet the crystalline substance itself may perhaps have been impure nicotonic acid contaminated with vitamine.

Sullivan and Voegtlin (506) fractionated wheat chaff and extracts of peas and ox liver. The residues from the respective alcoholic extracts were hydrolyzed with 5 to 10 per cent sulphuric acid for five hours in a stream of CO_2. The precipitates obtained with phosphotungstic acid were then decomposed, either directly or after dissolving in 50 per cent alcohol, with neutral lead acetate. After the elimina-

tion of lead, the filtrate was precipitated by an excess of silver acetate and treated with baryta, after removal of purine precipitates.

Steenbock (507) prepared from egg-yolk a substance soluble in water-acetone, active and not quantitatively precipitated by phosphotungstic acid. McCollum and Simmonds (l.c. 315) sought to concentrate vitamine B by extraction with various organic solvents. They found that beans could be extracted with ether, benzol, or acetone, without dissolving the vitamine. Subsequently it can be extracted with alcohol and the solution, when concentrated, may be taken up by benzol. The same method was applied to wheat germ and pigs' liver.

SYNTHETIC EXPERIMENTS

Although we undertook some synthetic experiments with nicotinic acid, based on its occurrence in the vitamine fractions obtained from various sources, we perceived that theoretical reasons for this trend of thought were hardly justifiable. Nevertheless, Williams (l.c. 473), instituted such experiments. He condensed p-oxy nicotinic acid with itself or with nicotinic acid, and believed that the first condensation product was much more active for avian beriberi than all other similarly synthesized condensation products. Thereupon, Williams (508) proposed the very attractive theory that oxy-pyridine and pyridine carbonic acid occur in two isomers, which possess different crystalline forms, and which may be transformed into each other under certain conditions. The labile form remained intact for some days and in this case was therapeutically active; then it changed into the inactive stable form. Later, Williams (509) extended his theory to include such pyrimidine and purine derivatives as were theoretically capable of forming the betaine ring. Williams and Seidell (l.c. 496) tried to convert adenine obtained from autolyzed yeast by adsorption with fuller's earth, by boiling with acetic acid and acetic anhydride, into an isomeric form. This labile form was supposed to have a therapeutic action; after recrystallization, this peculiarity was supposed to disappear, the substance being changed back again to the usual adenine. The theory of Williams explained the slight stability of vitamine B very beautifully, but unfortunately it could not be confirmed. Voegtlin and White (l.c. 497) sought in vain to produce an active product from adenine, while Harden and Zilva (510) tried to do the same with *o*-oxypyridine and adenine. Since the course of

reasoning pursued by Williams could not be confirmed, and since he has published no further related work since 1916, the subject may apparently be considered as closed.

STABILITY OF THE ANTIBERIBERI VITAMINE AGAINST HEAT AND CHEMICAL AND PHYSICAL AGENTS

As far back as the work of Grijns (l.c. 52) we knew that the vitamine B from Katjang-idjoe beans lost its curative properties on heating to 120° C. Ever since, a number of investigators have occupied themselves with this problem. It was soon evident that the stability was dependent upon a number of factors. Among these was the vitamine content of the starting material used, the chemical reaction of the substrate and the duration of the heating period.[4] Although these points are of great practical importance as regards the etiologic and dietetic significance, they are only of moderate theoretical interest, so long as the vitamine itself has not been prepared in the pure state and its characteristics determined. Only then shall we be in a position to conduct such experiments exactly. From the description of the isolation experiments, it is clear that this vitamine is quite stable and can withstand a number of chemical and physical manipulations. Therefore, it is hard to determine at which stage of the work this substance loses its activity. In the fractionation, there is very little loss of activity till we come to the last stages, where it decreases rapidly. One almost has the impression that the substances accompanying the vitamine serve to stabilize it. We must admit that we are still in the dark as to the cause, and although this may eventually find quite a simple explanation, it seems to us for the present remarkable and mysterious.

As regards the solubility of vitamine B, this has already been spoken of in the preceding pages. All investigators apparently agree that this substance is insoluble in strong alcohol. There are also some experiments which indicate that prolonged boiling in alcoholic solution may inactivate this vitamine. As to the effect of heat, the findings vary; similarly, with the effect of alkalis. On the other hand, the investigators are one in stating that this vitamine is very resistant to acid. Among other chemical reagents, diazotization, according to

[4] Emmett and Luros (l.c. 94) give a good bibliography relative to this question.

McCollum and Simmonds (l.c. 315), does not affect the activity of vitamine B, and this has recently been confirmed by Funk and Dubin (l.c. 493) who also showed that the substance stimulating the growth of yeast was not destroyed by reduction with palladium and hydrogen, as well as with zinc and hydrochloric acid; neither was it affected by oxidation with finely divided platinum and oxygen for 7 to 8 hours. Besides this, we (512) showed in 1916 that the action of radium in doses used in radium therapy is without destructive influence on vitamine B originating from autolyzed yeast. In accord with this, there is the work of Zilva (513) who investigated the effect of ultra-violet light on this substance. Contrary to the above findings, Sugiura and Benedict (514) report that large doses of X-rays of radium almost obliterates the vitamine of autolyzed as well as dried yeast. Weill and Mouriquand (515) carried out analogous experiments with Röntgen rays on barley kernels.

DEMONSTRATION AND ESTIMATION OF ANTIBERIBERI VITAMINE

At the outset of vitamine investigations, only one method was available for the demonstration of vitamine B. Beriberi was produced in chickens and pigeons (the latter being more commonly used) by feeding white rice, and then the preparation to be tested was given either per os or intramuscularly.[5] The latter method had the advantage of giving a more speedy effect and of making it certain that the solution was utilized by the animal, and had not trickled out of the beak. A negative finding of this therapeutic measure did not entirely indicate that the solution to be tested was inactive. Negative results may be due to the fact that the animal had progressed too far to be affected by the above measures or they might be due to the presence of toxic products. It was chiefly through the efforts of Osborne and Mendel, as well as McCollum and his coworkers, that the second method was then developed, of which we have already spoken in the chapter on the vitamine requirements of rats. This method consists in adding the preparation to be tested to a diet complete in everything but vitamine B. If growth was obtained with young rats, then the presence of this vitamine was

[5] We were one of the first to make use of the parenteral administration of this vitamine. Unfortunately, we have been unable to discover in the literature by whom this method was first introduced.

demonstrated. This method is more certain, though it has the drawback that much more time is required for its application. Both of these methods have the great disadvantage that in isolation experiments, every new fraction must await the findings of the animal experiments, the vitamine preparation in the meantime being subject to more and more decomposition. For these reasons, efforts have long been made to develop a method that would permit of conducting a test in the shortest possible time. For practical reasons too, such a method would be of advantage in determining the vitamine content of various foodstuffs.

The first step in this direction was made by Fraser and Stanton (l.c. 55). Based upon Schaumann's theory of the lack of phosphorus, they believed that the vitamine content of rice could be estimated by the amount of phosphorus present. Voegtlin and Myers (516) also suggested the phosphorus content of wheat and corn as a somewhat reliable indication of the amount of vitamine contained therein. On the other hand, Green (517) showed that if the phosphorus content of American corn is taken as a unit, then all South African varieties of corn, which are very poor in phosphorus, might be regarded also as very poor in vitamines, which is, however, not the case. Ottow (518) is likewise of the opinion that the estimation of the phosphorus content alone may lead to false conclusions. He believed that the determination of the quantity of alcohol-soluble fraction of rice was more reliable, at the same time regarding the use of animal experiments as the only method to be relied upon.

We (519) sought, at first, to form some conception as to the amount of vitamine present, by determining the nitrogen in foodstuffs having a slight non-protein nitrogen, such as milk. For this purpose, we dried some milk, made an alcoholic extract of the portion precipitable by phosphotungstic acid, and analyzed it for its nitrogen content. The only result apparent was that on centrifuging the milk, a large part of the residual nitrogen is lost and evidently goes over into the cream. Whereas in a non-centrifuged milk this fraction amounts to about 2.2 mgm. per liter, in the centrifuged milk it is only 1.4 mgm. This shows perhaps that milk fat (butter) may contain nitrogenous vitamine. Furthermore, since we noted that vitamine fractions contained substances whose nitrogen could be only partially estimated by means of a Kjeldahl determination, we wanted to make use of the difference between the analyses, according to

Dumas and to Kjeldahl, as an indication of the vitamine fraction. However, since this fraction contained a relatively large amount of pyridine derivatives, the method is valueless so long as we do not know the relationship between these substances and the vitamines. Brill and Alincastre (520) tried, in this way, to average the maximum vitamine content from the phosphotungstic acid precipitates of various vegetables. Seidell (521) sought to determine the vitamine content of yeast by estimating the nitrogen in activated fuller's earth. Eddy (522) tried to do the same with an activated fuller's earth out of pancreas. Since fuller's earth adsorbs also a large amount of inactive material, this method is of no special consequence. In discussing the chemistry of vitamine B, it will be recalled that we have often made use of a reaction introduced by Folin and Macallum (l.c. 486) for the determination of uric acid and phenol. It was shown by us, and later by others, that all vitamine-containing extracts give this reaction. It depends upon the development of a blue color with phosphotungstic and phosphomolybdic acids on the addition of sodium carbonate. The chemical nature of the substances giving this reaction was investigated by Funk and Macallum (523), as well as by Lewis and Nicolet (524). With Macallum, we also showed that if an incision is made in the maize kernel and the test applied in situ the blue color develops in the part known to be rich in vitamine. Naturally, these parts are even richer in other extractives. As this method came into use for the chemical analysis of various corn meal products, we (525) showed that in extracts, prepared either cold or warm, the developed color increased with the vitamine content. In the warm extracts, the color was weaker, showing that the substances responsible for the color reaction are destroyed by heat. If it should appear later, with greater certainty, that this reaction has nothing in common with the vitamine, it might still be useful in vitamine fractionation as an index of the purity of the isolated substances. These reactions could conceivably also serve as an index of the purity of the isolated lipoids.

Green (526) tried to estimate the vitamine content of various foodstuffs by means of a biological method (animal experiments). The method depends on the length of time pigeons survive on various diets, rich or poor in vitamines. As a result of these studies, Green proposed the following formula:

$$S = \frac{C}{V - X} \cdot \frac{1}{K}$$

in which S is the duration of life; C, the amount of vitamine that the animal may lose without developing beriberi; V, the amount of vitamine necessary to provide for the metabolism of a given quantity of food; X, the quantity of vitamine in the given diet; K, the constant depending upon the quality and quantity of the diet fed. By means of this formula, the vitamine content of various foodstuffs, especially corn, was determined. The figures obtained correspond in general with experience gained by other investigators with the same products. The weak point of the formula is that it shows the presence of vitamine in white rice despite all evidence to the contrary. Aside from this, it is obvious that the survival of pigeons depends on many unknown factors.

We come now to the consideration of a method, briefly discussed in the chapter on the vitamine requirements of yeast, which was indicative of real progress. This method is based upon the fact that certain yeasts require an addition of substances containing vitamines in order to grow. We have already spoken of the work of Williams (l.c. 128), Bachmann (l.c. 129) and Abderhalden and Schaumann (l.c. 499)—all of which led to the development of this method. Soon after, papers were published on the practical application of the method. Eddy and Stevenson (527) tested the method of Bachmann and found that it was not so very reliable. Thereupon they turned to the method of Williams, by introducing exactly calibrated micropipettes (similar to those used in opsonic index determinations) for drawing up the yeast cell suspension and the vitamine solution, which made the method somewhat more exact. The yeast and vitamine units, with the necessary controls, were deposited on a counting slide and kept in the incubator. After a certain time, the slides were removed from the incubator, stained and the cells counted. The objection to this method is the fact that clumps of yeast cells are often introduced with the cell suspension. In spite of shaking the yeast cell suspension and partial centrifugation to remove the clumps, this objection was not quite overcome and the results were therefore uncertain. Among other substances, Eddy and Stevenson also tested the products isolated by us from yeast in 1912 and 1913 (see chapter on the Chemistry of Yeast) and found them active, while nicotinic acid was inactive. The active substance was not destroyed by heating to 100°C., but partially so at 120°C. Using this method, more vitamine was found in the mammary vein than in the jugular.

The methods of R. J. Williams (528) and of Funk and Dubin (l.c. 511) then appeared almost simultaneously, both signifying a step forward in the demonstration of the part played by the vitamines in yeast growth. The method of Williams is as follows: 0.3 gram of ordinary fresh bakers' yeast was removed from the center of a yeast cake and suspended in a liter of sterile water. Of this suspension, 1 cc. was introduced into the nutritive solution and the cultures kept in the incubator at 30°C. for eighteen hours, after which growth was stopped by the addition of formalin. The yeast cells were then

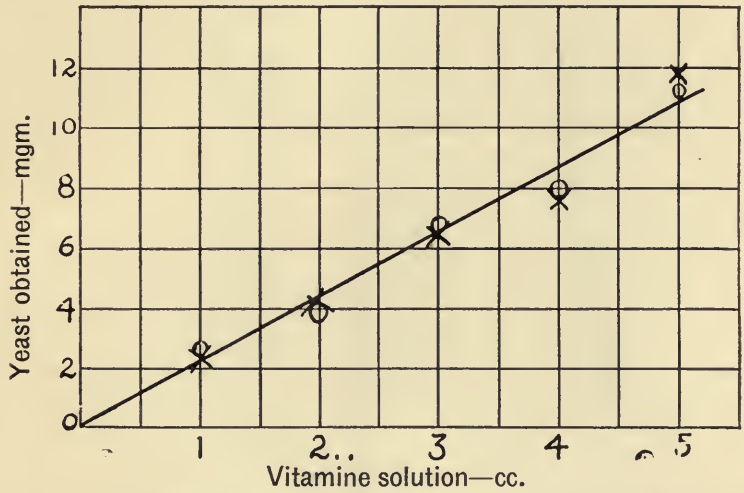


FIG. 47. INFLUENCE OF VITAMINE B CONCENTRATION ON YEAST GROWTH (WILLIAMS)

filtered on a Gooch crucible, washed with water and alcohol and dried at 103°C. for 2 hours. With a vitamine solution of a source not mentioned, a growth curve was obtained, reproduced herewith. He showed that with higher vitamine concentrations, the quantity of cells rose proportionally and that under certain conditions, the curve approaches a straight line. Williams also observed that certain extracts such as autolyzed yeast, or extracts heated to a high temperature in the presence of acids, could exert an inhibiting influence on the growth of yeast. In such cases, an abundant dilution is of great help and yields better results.

The method of Funk and Dubin is still more simple and is briefly as follows: A yeast suspension is prepared by shaking 100 cc. of Nageli solution inoculated with a platinum loop-full of a 48 hours pure yeast culture, preferably bottom yeast. Two test tubes are

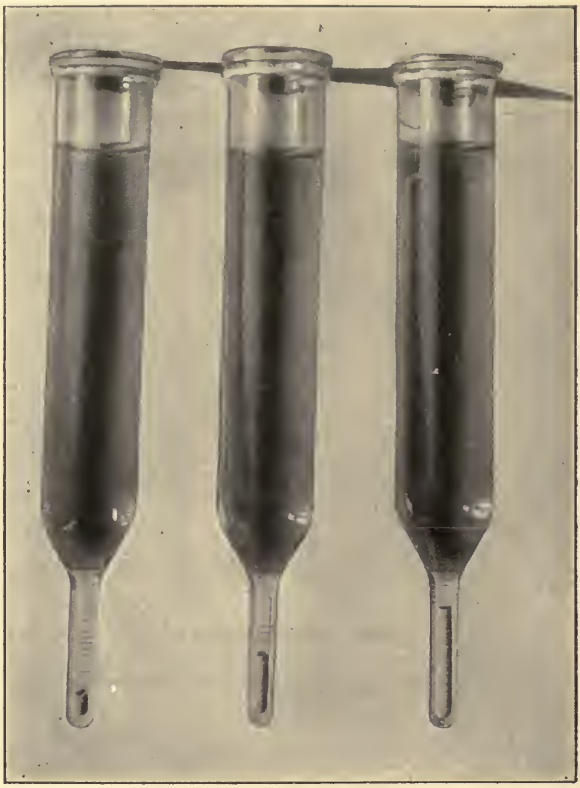


FIG. 48. CENTRIFUGE TUBES USED IN THE DETERMINATION OF THE EFFECT OF VITAMINE ON YEAST GROWTH

The tube at the left shows the reading of the blank, while the other two tubes show the readings obtained with different amounts of autolyzed yeast (Funk-Dubin).

prepared, containing the unknown vitamine solution to be tested; in addition, there are two controls—one with yeast suspension alone, and one with the vitamine solution alone. The last control is not very important and serves only to detect the formation of a pre-

cipitate or cloudiness at the beginning of the test. All of the test tubes are placed in the incubator for 20 hours at 30°C., after which the cells are inactivated by immersing the tubes in a water bath at 70 to 80°C. for a few minutes. The contents of the tubes are then quantitatively transferred to centrifuge tubes, the lower part of which is in the form of a capillary, graduated in mm. The tubes are centrifuged for 15 minutes at about 2600 r.p.m., and the volume of cells read on the graduated capillary of the centrifuge tube. The reading should be taken at once, since there is a tendency for the yeast cells to swell up, after a time. The control, without the addition of vitamine, must be subtracted from the reading.

We found that the controls with yeast alone were quite constant and almost independent of the number of yeast cells in the suspension

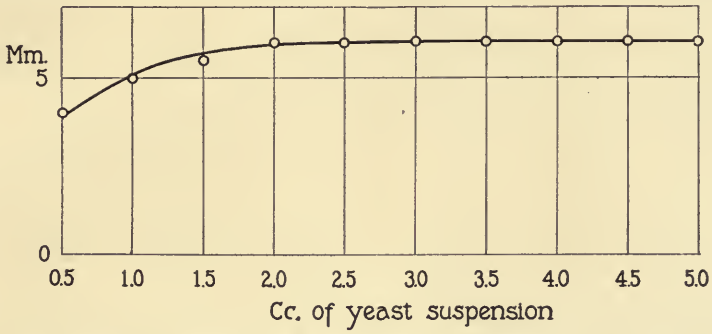


FIG. 49. EFFECT OF VARYING AMOUNTS OF YEAST SUSPENSION (FUNK-DUBIN)

used, as may be seen from the curve. Experiments with increasing amounts of autolyzed yeast gave a curve markedly different from that of Williams, but which is perhaps better applicable to the study of natural extracts. We feel that our results have been considerably influenced by the presence of inhibiting substances. For this and other reasons, it appears somewhat premature to estimate the vitamine content of various foodstuffs by the use of these methods.

A number of problems were undertaken with the help of the above method, and we shall record some of the results. We showed that analyzed substances, isolated from yeast seven years ago by the author, still retained a definite activity, although they had been recrystallized to constant melting point. On the other hand, nicotinic acid obtained from yeast was inactive. White polished rice

showed no influence on yeast growth; this was also true of vitamines A and C (after vitamine B had been completely removed by adsorption with fuller's earth). The active substance was found also outside of the wheat and corn germ, though in smaller amounts. The substance influencing the growth of yeast was very resistant towards heat; it could be diazotized, oxidized and reduced without loss of activity. With this method as a control, a fractionation of yeast was undertaken; partial results have already been published (Funk and Dubin (l.c. 511)) but the work is not yet completed.

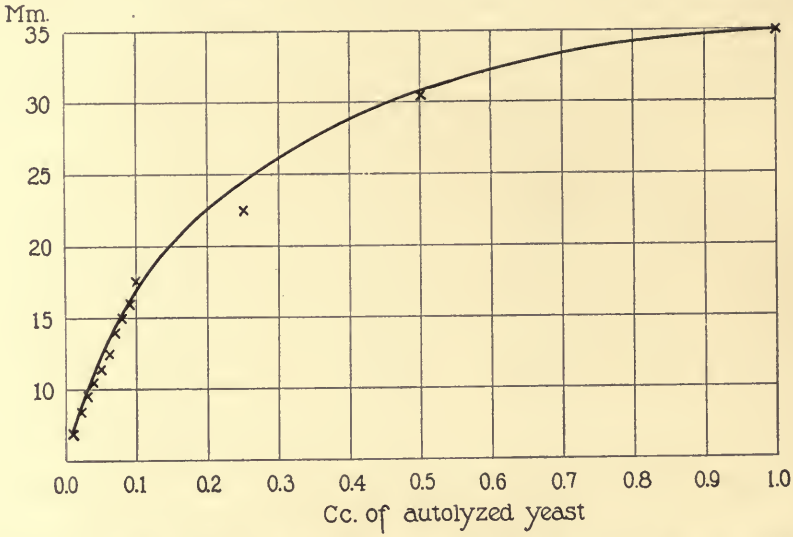


Fig. 50. Vitamine Activity Shown by Increasing Amounts of Autolyzed Yeast (Funk-Dubin)

The use of these methods was criticized by de Souza and McCollum (529). They employed the first method of Williams, slightly modified, for their investigations and found, as a rule, that with increasing amounts of vitamine B, the growth of yeast is favorably affected. They believed, however, that the method could hardly be applied to the determination of vitamine B, because glucose and amino-acids also show a growth-promoting action. Despite this, if we examine their findings more closely we see that the addition of a 10 per cent glucose solution had no more influence during the first 24 hours, as compared with the controls; it was only after 48 hours—

and not always so—that some effect was noticeable. This protraction of the influence showed that the accelerative action of glucose was of a secondary nature, and might perhaps be explained by the plasmolysis of some yeast cells. When de Souza and McCollum speak of the favorable action of amino acids, they mean by that a product resulting from the sulphuric acid hydrolysis of meat. This preparation, as well as an alcoholic extract of wheat germ, was shown to be free from vitamine B in rat experiments, while they were still active for yeast. Hydrolyzed meat gave only double the amount of cells, so that the action was not so pronounced. These investigators admitted that their experiment did not demonstrate that vitamine B played no part in the growth of yeast, they likewise showed that the growth of rats and yeast does not go parallel with the same extracts. The question as to the identity of vitamine B with the substance, promoting the growth of yeast will be discussed in the next section.

Newer investigations have shown that the yeast test is of value in determining the relative richness of extracts in water-soluble vitamines, but cannot be used for the quantitative determination of vitamine B.

THE POSSIBLE IDENTITY OF VITAMINE B WITH THE SUBSTANCE STIMULATING THE GROWTH OF ANIMALS AND OF YEAST

We have already (p. 41) touched upon the question raised by Mitchell (l.c. 93) as well as by Emmett and Luros (l.c. 94), as to the identity of these substances. As regards the comparison of vitamine B with the substance promoting the growth of rats, Funk and Macallum (530) showed that the latter substance could be precipitated with phosphotungstic acid, whereupon further fractionation was tried. Since the diet we used at the time did not contain sufficient vitamine A, the results were not particularly fruitful, and seemed to indicate that pigeons require less vitamine B for protection against beriberi than do rats for growth, or that vitamine B is more stable than the growth vitamine. Drummond (l.c. 498) corroborated our findings and came to the conclusion that, in all probability, both substances are identical. Emmett and Luros (l.c. 94) seem to have a different opinion on this matter. They investigated the effect of autoclaving at various temperatures for various periods of time. On the one hand, these products were given, as such, to

pigeons and on the other, together with lactalbumin, butter, salts and lard, to rats. Vitamine B, which is necessary for pigeons, appeared to be unstable and was totally destroyed by heating for 2 hours at 120°C., while rats could still grow on the above food mixture. Emmett and Luros came to the conclusion that these vitamines are different from each other. Nevertheless, we saw in our investigations on yeast, contrary to the above experiment, that vitamine B was resistant to heat; furthermore, in the experiments of these workers one kind of animal was fed rice alone while the other received various additions. As we shall see later, protein may easily take the place of a vitamine addition, besides which, these animals may differ with respect to their vitamine requirements.

As regards the identity of the growth substance for yeast with vitamine B, the question is of particular importance since we must know, when using the yeast method described in the previous chapter, which substance we are determining. There is no doubt, however, that the substance important for the growth of yeast is much more stable to heat, and yet we saw in the chapter on the chemistry of vitamines, that vitamine B resists many manipulations, and still retains its activity after many weeks of work. Emmett and Stockholm (531), on the contrary, saw that strongly heated unpolished rice, although inactive for pigeons, is just as active as the natural product for yeast cells. The same findings were obtained with filtrates resulting from the treatment of extracts with fuller's earth. In these cases, the filtrates were active for yeast but not for rats.[6] Emmett and Stockholm believed that further research must establish either the identity or dissimilarity of these substances, and it would not be correct in the meanwhile, to regard the yeast method as suitable for the determination of vitamine B.

Recently, Funk and Dubin (l.c. 129d) have corroborated the statement of Emmett and Luros that the yeast substance is not identical with vitamine B. If autolyzed yeast is shaken with a small amount of fuller's earth vitamine B is usually removed almost quantitatively. Now if the filtrate from the above is repeatedly shaken with larger amounts of fuller's earth, the substance acting

[6] Karr (l.c. 404), working on dogs, noted that vitamine B of yeast is very stable. Autoclaving for 3 to 4 hours with subsequent heating at 108°C. for 72 hours destroyed only a part of this substance.

on yeast—and which we call vitamine D—is likewise almost completely removed. We have not succeeded thus far in separating D-vitamine from B-vitamine quantitatively. The animal experiments performed thus far in which vitamine B preparations were used have to be repeated, since such preparations contain at least two vitamines—B and D. While pigeons get along very well on vitamine B alone, rats require vitamine D besides vitamine B. Other animals are being investigated in this respect.

Vitamine D is one of the substances necessary for the growth of certain bacterias, as we have already pointed out. The isolation has been attempted by Goy (531a) who thought it was a non-nitrogenous substance but the pure substance he isolated proved to be inactive. The isolation also was attempted by Fränkel and Schwartz (531b).

We also feel that this subject needs further investigation. For in every one of the cases investigated, we tested the activity after the naturally occurring substances had been altered by the manipulation undergone; we do not know what the action of the substances in the natural state would have been. It may, for example, be established later that vitamine B is a uniform substance, but that parts of it may be utilized differently by yeast, pigeons and rats. The analogy between the three modes of action, in relation to its occurrence in Nature, is so far extended that we are perhaps justified in attributing to it a genetic relationship. The findings of Funk and Dubin (l.c. 493), and others, as to specificity, also point to this conception.

SPECIFICITY OF ANTIBERIBERI VITAMINE

A short time after the appearance of our chemical vitamine studies, a number of publications appeared describing the curative action on beriberi of various quite heterogeneous substances. These papers had the regrettable effect that doubt arose as to the specificity of vitamine influence. It almost seemed as though practically all chemical groups, under certain conditions, could act as vitamine. Although most of these results were either disproven or else abandoned by their respective authors, we see unfortunately that these results gained acceptance in the literature. We must not forget that the cure of acute beriberi symptoms may be accomplished theoretically in two ways, first, by the addition of the specific vita-

mine, second, by measures which will suddenly mobilize the reserve vitamine present in organs and tissues. As regards spontaneous cures, which we personally have not seen (but which seem to be plausible), occurring ostensibly in starvation, we may explain them by the catabolism of the tissues and the consequent liberation of the vitamine. Every substance, having pharmacological action, which stimulates metabolism may also contribute to the above-mentioned action.

Eijkman (532) stated that pigeons, used by us for the demonstration of vitamine B, were not suitable for this purpose, since they show a different behavior from chickens. He maintained that a mixture of potassium chloride and sodium chloride (3:1) in doses of 20 to 40 mgm. can cure beriberi in pigeons, but not in chickens. We (533) tested this out on pigeons, but could not corroborate Eijkman. In spite of this, we note that his findings are frequently quoted in the literature. Later, we showed (534) that certain purine and pyrimidine derivatives exerted a life-prolonging action on pigeons fed on rice. This observation was confirmed by some, Williams and Saleeby (l.c. 472), and refuted by others. Since these products are frequently not indifferent pharmacologically, their behavior, as described above, may perhaps be explained in that light. Cooper (535 and 536) found that quinine and strychnine exhibited a definite action on experimental beriberi. Later, he showed that quinine contained vitamine as an impurity; as for the action of strychnine it may be explained by its effect on the metabolism. Abderhalden and Lampé (l.c. 25) stated that castor oil had a curative effect, but later on Abderhalden and Ewald (537) reported that they could not confirm the earlier finding. They believed, however, that vitamine B was analogous to B-imidazolethylamine. Dutcher (l.c. 276) investigated the behavior of thyroid, thyroxin (chemically pure active substance of thyroid), tethelin (lipoid from the hypophysis) and pilocarpine and found that these substances have a therapeutic effect on beriberi. Pilocarpine was found inactive by Abderhalden and Ewald; thyroid was regarded as inactive by Vedder and Clark (l.c. 266). Seaman (537a), on the contrary, could demonstrate the presence of vitamine B in thyroid. Inasmuch as thyroid substance acts as a powerful stimulus to the metabolism, the mobilization of the vitamine from the tissues may, under certain circumstances, simulate a curative action, although we convinced ourselves that

death is hastened because of it. Regarding the action of tethelin, when one considers its method of preparation, it may contain vitamine as an impurity. Dutcher, Holm and Bierman (511) tested various indol derivatives of thyroxin and found them active, but their findings require further corroboration. To complete our data on this question, we may mention that it was impossible for Chamberlain, Vedder and Williams (l.c. 64) and Abderhalden and Schaumann (l.c. 499) to influence beriberi by the administration of known substances, and especially a mixture of amino acids. Viewing all statements to the contrary in a critical light, we must recognize that vitamine B has a well defined and specific action, and it is not to be substituted by other products.

PHYSIOLOGY AND PHARMACOLOGY OF ANTIBERIBERI VITAMINE

To draw a general picture of the nature of beriberi, we see, first of all, a very definite retardation of the life processes,[7] similar to that observed in inanition. In this respect, Ramoino (538) showed that in rice-fed pigeon, the respiratory quotient is very low but may be raised again by an addition of vitamine. Similar observations were made by Jansen and Mangkoewinoto (539). This is evident also in McCarrison's (l.c. 300) investigations of avian beriberi, in which he described pathological changes in the intestines and some glandular organs. In hunger, however, the whole organism is attuned to endure the least possible loss of energy and substance. On the other hand, in experimental beriberi only a diet free from or poor in vitamines is fed. In this way, energy is expended, digestive juices are secreted and the assimilative processes are continued. At present, we are not quite in a position to set forth the primary causes of the whole disturbance. It is not yet possible to say, of the pathological conditions observed in this disease, which may be regarded as primary and which secondary. We only know that the animal requires a minimal amount of vitamine in order to regain its normal condition. With one stroke, the entire condition is altered, and it can hardly be believed that this is brought about by such a small quantity of

[7] In accord with this, there are the observations of Drummond (l.c. 33 and Abderhalden (l.c. 500) on the subnormal temperatures of beriberi pigeons.

substance.[8] In order to find an analogy, we may compare an animal on a vitamine free diet with a machine, running without oil. At first the machine operates faultlessly, but gradually trouble arises and then, in due time, greater defects occur which can nevertheless still be corrected by proper oiling. In the end, a condition results which can no longer be be remedied and the machine falls to pieces. In animals, we know only the primary cause, lack of the specific vitamine, similar to the oil in the above mentioned analogy. It still seems tempting to attribute all of the observed phenomena to changes in the central nervous system, but another explanation is that the vitamine might be thought of as the mother substance of an important product of the endocrine glands—a conception which we, among others, thought to be plausible and which has again been put forward by Massalongo (540). Another relationship may be seen in the origin of the digestive juices. Lumière (541) believed that the primary cause of beriberi is to be noted in the lack of secretions. The same view was held by Damianowich and Pilado (541a). Other investigators sought for a relationship with the oxidation processes in the body. Dutcher (542) and Dutcher and Collatz (543) demonstrated a decrease in the catalase in the organs of beriberi pigeons, but almost the same thing was described in starving animals by Burge and Neill (544). Particularly in the observations as to the nature of beriberi, is the picture of this disease somewhat obscured by complication with symptoms of inanition. Jansen (545) studied

[8] The influence of small quantities of vitamines on the metabolism of pigeons is well illustrated in the metabolism studies of Schaumann (l.c. 403).

	VOLUNTARY INGESTION OF WHITE RICE	CALORIES	LOSS OF WEIGHT	FECES / FOOD	PERCENTAGE RELATION OF OUTGO TO INTAKE				
					N	P_2O_5	Ash	CaO	MgO
	grams		*per cent*	*per cent*					
Without vitamine.....	204.3	658.7	20.7	11.47	69.3	45.26	40.13	36.99	238.89
With vitamine...	295.1	951.6	9.41	8.53	101.4	63.56	62.12	118.9	347

The nitrogen balance was positive and the balance of the inorganic constituents was likewise improved. The weight of the feces, in comparison with the food intake, showed that the food was utilized much better.

the question as to the identity of secretin with vitamine and came to the conclusion that they are not identical. Voegtlin and Myers (546) also took up this question and showed that both substances occur in the same fraction; they believed that both substances at least resemble each other, if they are not entirely identical. The results of their experiments do not seem to lead to such conclusions, since they noted in the same fraction a strong secretin and a weak vitamine action, whereas a vitamine preparation showed a considerable vitamine and only a slight secretin content. In this connection, they showed later that vitamine fractions were contaminated by a histamine-like substance. That the substances are not identical was shown by the work of Cowgill (546a) and of Anrep and Drummond (546b). Bickel, Eisenhardt and Djenab (547) have demonstrated the presence of a secretin in spinach, which stimulates the stomach juice and pancreas secretion. The substance occurred in the arginine-histidine fraction and was inactivated by heating to 140°C. It occurred in combination and could be liberated by hydrochloric acid hydrolysis. The relationship of this substance to vitamines was discussed by the authors, and their results were confirmed by van Eweyk (548).

Boruttau (549) believed that yeast, or the aleurone layer of oat kernels, contains a substance which acts as a specific antidiabetic, since their extracts greatly decreased the output of sugar. Uhlmann (550) investigated the pharmacological action of orypan (extract of rice polishings), as well as the extracts of various vegetables and foodstuffs. The action noted was similar to, but not identical with, that of choline, and pilocarpine. Uhlmann ascribed this to the presence of vitamine. It is nevertheless apparent that in his cases, he was dealing with histamine-like substances. A vitamine preparation made by us, was shown by Prof. Cushny to have no action on blood pressure, respiration and the heart. No relationship to the endocrine glands, aside from that already described in avian beriberi, was noted.

When an animal is fed a vitamine-free food, what happens to the vitamine reserves stored up in the organs? It would be plausible to assume that the animal would hold on to this valuable substance, but this is not the case. Cooper (l.c. 536) showed that when feces of chickens, fed on unpolished rice, or of rabbits, fed on bread and cabbage, are extracted with alcohol and the concentrated extracts

given to beriberi pigeons, a cure could be obtained. This shows that all the vitamine of the food is not assimilated, but that a portion of it appears in the feces. Another explanation might be found in the vitamine content of the bacterial intestinal flora, although Braddon and Cooper (551) noted only a small quantity of vitamine in these bacteria. Portier and Randoin (l.c. 217) believed on the contrary that the vitamine of the intestinal canal arises from the bacteria. Muckenfuss (552) found vitamine B in bile and urine, and was confirmed, as regards the latter, by Gaglio (553) and by Funk and Dubin. Funk showed that when pigeons, which had died of beriberi, were minced, extracted with alcohol, the extract concentrated, the residue taken up with water and given to other beriberi pigeons, the latter were promptly cured. This experiment, corroborated by Theiler, Green and Viljoen (l.c. 278) showed definitely that animals develop beriberi in spite of the fact that they still have vitamine in their tissues. It seems that a portion of the vitamines in the tissues (perhaps as a constituent of the cell protoplasm) is so combined that the animal itself cannot utilize it. Another explanation of this is to be found, perhaps, in the work of Green on pigeons (l.c. 526); he noted that the animal organism requires a rather definite amount of vitamine for normal health. This really seems to be the case, and as the quantity drops below the minimal need, it leads to an avitaminosis. The quantity of vitamines that may be liberated from the organs normally is very small. If the animal is given a diet poor in vitamine, it might be thought that the animal would consume more of the food in order to obtain the necessary vitamine; but this is not the case. It was supposed by Osborne and Mendel (554) that some foodstuffs contain sufficient vitamine for rats, if these animals would but ingest more of this food mixture. But it is right here that we meet with a difficulty that is also of practical importance, namely, the question of vitamine dilution in the natural foodstuffs. With every diet prepared, the needed amount of vitamine must be supplied if the diet is to prove satisfactory.

THE INFLUENCE OF DIETARY COMPOSITION ON THE VITAMINE REQUIREMENT

Regarding the influence of a large or small addition of a vitamine-free diet on the occurrence of beriberi, the literature is full of conflicting opinions. While Maurer (555) and Cooper (l.c. 536) stated that

the addition of large quantities of rice hastens the onset of the disease, Chamberlain, Bloombergh and Kilbourne (556) noted that the disease developed sooner on a smaller rice feeding. They found, in fact, that beriberi arises in hunger. These observations were later corroborated by Eijkman and Hoogenhuyze (557). They found that the outbreak of beriberi could be obtained by starvation, especially when the precaution was taken to wash out the organism with water. The starvation beriberi resulting thereby could be cured on the addition of vitamine. At all events, Theiler, Green and Viljoen (l.c. 278) noted spontaneous cures in animals receiving only water. These therapeutic results were attributed to the mobilization of the vitamine present in the tissues. Walshe (558) found no beriberi in chickens that had been starving a long time; these observations have not yet been made on pigeons, and it is an open question as to whether starvation beriberi may develop in other animals, and also in man.

The influence of the composition of the diet on the occurrence of beriberi was investigated by the author (559) in 1913. At that time, there appeared the paper of Abderhalden and Lampé (l.c. 25), in which they formulated the "toxic" theory of beriberi. The symptoms of the disease were apparent later on cooked rice than on raw rice. This observation, which in itself was correct, but which was thought to be due to the elimination of toxins, may be explained in a very simple way. Cooked rice takes up so much water that it is impossible to feed an amount corresponding to uncooked rice, and the results appear as follows:

COOKED RICE		RAW RICE	
Appearance of beriberi	Dead	Appearance of beriberi	Dead
days	*days*	*days*	*days*
44	44	25	26

However, if pigeons were fed cooked rice corresponding to 10 grams of raw rice, then the results were somewhat different:

COOKED RICE		RAW RICE	
Appearance of beriberi	Dead	Appearance of beriberi	Dead
days	*days*	*days*	*days*
27	29	28	30

Here, we see that the disease develops almost simultaneously when comparable amounts of rice are fed. Later, we (560) showed that when pigeons are fed 5, 10 and 20 grams of rice daily that the disease develops the sooner, the more rice is fed:

	AMOUNT OF RICE		
	5 grams	10 grams	20 grams
	days	*days*	*days*
Beriberi	39	36	22
Dead	42	38	22

We also tested this observation on a synthetic diet, the pigeons receiving equal quantities of food (12.5 grams) in every case:

DIET	SALTS	CASEIN	SUGAR	LARD	STARCH	BERIBERI
A	4	60	12	12	12	30 days
B	4	12	12	60	12	40 days
C	4	12	12	12	60	24 days
D	4	12	60	12	12	28 days

From these findings we concluded, at that time, that vitamine B plays a particular part in the carbohydrate metabolism, since on feeding large amounts of carbohydrates, the pigeons developed the disease sooner. Our observations on the influence of rice feeding were confirmed by Braddon and Cooper (l.c. 551 and 561) in 1914, by Weill and Mouriquand (562) and also by Green (l.c. 526). Green, however, assumed that not only carbohydrates but the entire metabolism plays a rôle in the vitamine requirements. However, other investigators were not in accord with our views. For example, Eijkman and Hoogenhuyze (l.c. 557) found that an addition of meat did not prevent the appearance of beriberi symptoms. Inasmuch as this disease may occur likewise on an exclusive meat diet, they used this in combating our views. Since about 40 per cent of the protein in the body is transformed into glycogen, and since the destruction of the tissues proceeds similarly in starvation, these facts, in reality, cannot be used to discredit our findings. Vedder's (563) conclusions were similar to those of the Dutch investigators. Altogether he carried out five experiments, which are perhaps less convincing than appears at first sight.

In the first experiment he fed 11 chickens, varying in weight between 1800 and 3800 grams on rice, which they ate voluntarily; the uneaten rice was weighed the next day. No relationship was evident between the appearance of beriberi and the amount of rice utilized. Since the birds varied considerably in age and vitality, so that the energy expended and required were different, and since this experiment was carried out on two series of experimental animals, in two different months, with a possibility of the dissimilarity of the external temperature, therefore this experiment is not quite significant. In a second experiment, Vedder fed forcibly 100, 50, and 25 grams of white rice daily to chickens averaging 1200 grams in weight. Vedder then stated that the pigeons could die as well from over-feeding as from underfeeding, because rice, collecting in the crop, mechanically caused disturbances not related to the stimulation of metabolism. If we assume, with Vedder, that chickens fed 100 gm. of rice really die as a result of over-feeding, and only consider the birds receiving less rice, then chickens on 50 grams of rice developed beriberi in 20 days and those on 25 grams of rice, much later, which naturally completely corroborates the results of other investigators. In a third experiment, unpolished rice was used, but in this case, the animals did not die as a result of over-feeding, although they were given 50 and 75 grams of rice daily. In a fourth experiment, chickens were fed on sterilized meat and eggs, and the results show definitely that eggs contain some vitamine while meat contains a greater quantity. Vedder believed that if beriberi could occur in the absence of carbohydrates, then it was certain that vitamine B played no part in the metabolism of this dietary component. We have already seen that this conclusion is, in all probability, incorrect. If these foods were vitamine-free, the retardation of the beriberi symptoms would be the best demonstration for the correctness of our observations. In the fifth and last experiment, chickens were fed on sterilized eggs and meat, with the addition of white rice. The eggs, apparently egg-white and egg-yolk, were evidently not entirely vitamine-free, and the residual vitamine might perhaps suffice for 25 grams of rice. The eggs and meat used here, according to Vedder's statements, were supposed to be identical with the preparations used in the fourth experiment. This is very unlikely, since the chickens, in the fourth experiment, developed beriberi in 61 days, when fed on 50 grams of eggs, while in the fifth experiment, the animals developed no beriberi symptoms on an addition of 25 grams of eggs. As we see from the above, Vedder's objections do not carry any weight. Aside from this, upon examining our pigeon experiments more closely and disregarding those fed on larger quantities of rice, the results on lesser quantities of rice still prove our contention. The amount of food was kept constant (12.5 grams daily) in another experiment, where a synthetic diet was used which confirmed our rice results, so that the food only varied qualitatively but not quantitatively.

The chief difficulty encountered in this experiment was that the amount of vitamine needed for metabolism depends not on the ingested quantity of food, but on the portion assimilated. This con-

dition is controlled with difficulty in pigeons and chickens, because of the scattering of the food and the ease with which they vomit.

It would, therefore, be desirable to repeat these experiments on rats, in which it is easy to control the food. Such experiments, which were carried out by Funk and Dubin (l.c. 331), and which confirmed in general the results with pigeons, will be described in another chapter. Whether our view, that vitamine B plays an important rôle in carbohydrate metabolism, may be regarded as correct must remain for the future to show, since simultaneously with an enrichment of the carbohydrates in the form of starch or sugar, there is a decrease in protein. That protein plays a particular rôle in nutrition, is well known. Maignon (564) fed rats on diets which varied considerably in their protein, starch and fat content, but unfortunately the vitamines were not provided for. While each of the protein-fat combinations could maintain the animal at a constant weight for 50 days, this was true of the protein-starch mixture only when both components were present in equal quantities. From this, it was concluded that protein is better utilized in the presence of fat than in that of starch. The best nutrition was observed when a mixture consisting one one part protein, one part fat and 1.33 parts starch was fed. Bierry and Portier (565) obtained similar results, and expressed the view that there must be a very definite relationship between the protein and fat of any diet. Bierry (566) noted that disturbances in metabolism could be prevented only when the individual dietary constituents were present in proper proportions.

While we believed, at first, that the delaying effect of a protein addition on the development of beriberi was due to the presence therein of vitamine B, this view is perhaps no longer tenable. We shall see later that the "vitamine sparing" action of protein, or the presence of a special substance essential to life may play a part in this phenomenon. For this reason, we must be conservative with our conclusions when extra protein is added to the diet. To illustrate, let us consider the work of Johns and Finks (567), who investigated the nutritive value and B-vitamine content of war bread, which was milled to the extent of 74 per cent. Working with rats, they found that the above food contains sufficient B-vitamine but not enough protein, since the animals resumed normal growth on the addition of casein. This conclusion is perhaps not quite correct, for the growth-inhibiting influence may possibly still have been due

to a lack of vitamine B. Similar conditions are frequently met with in the study of pellagra and hunger edema, to which we shall refer again in the chapter dealing with the question. We have already seen in the investigation of Funk and Dubin (l.c. 331) that a larger addition of carbohydrates increased the vitamine B requirements. In this respect, rats are similar to pigeons except that in rats, an excess of fat causes much greater disturbances than in pigeons.

Besides the above mentioned results, Funk and v. Schönborn (l.c. 304) found that whereas in normal pigeons, the glycogen and blood sugar content of the liver is 1.17 per cent and 0.1 per cent, respectively, on an artificial vitamine-free diet, the glycogen content drops to 0.48 per cent and the blood sugar rises to 0.15 per cent.[9] Pigeons fed on an excess of sugar showed 4.5 per cent glycogen and 0.15 per cent blood sugar. On an excess of starch, there is no glycogen and 0.26 per cent blood sugar. In the last case, if vitamine B is added, the values for glycogen and blood sugar approach normal. These results were later repeated several times and corroborated by the author (568).[10]

Besides this, we also studied the influence of substances having a known action on carbohydrate metabolism. Among them, the effect of glucose, phlorizin, adrenaline, pituitrin, thyroid and parathyroids on pigeon beriberi was tested, normal and rice-fed pigeons being used. Of special interest, was adrenaline, which shortened the life of the animal; this was also true of thyroid. The influence of parathyroids was quite different. While thyroid raised the blood sugar content and the amino nitrogen in the serum, and lowered the glycogen content of the liver, the addition of parathyroids increased the glycogen and amino acid content, but had little effect on blood sugar. We must admit, however, that Elias and Kolb (569) also demonstrated glucosuria in starving dogs, which was attributed to acidosis, and which could be remedied by the administration of alkali. We have already emphasized that it is not known, at present how many of the starvation symptoms may be regarded as results of

[9] Scott (567a) has found the normal blood sugar content in pigeons higher, but the difference may be due to a different method of bleeding the animal, a fact which has been frequently observed by us.

[10] The finding of glucosuria in pigeon beriberi has not yet been confirmed in human beriberi.

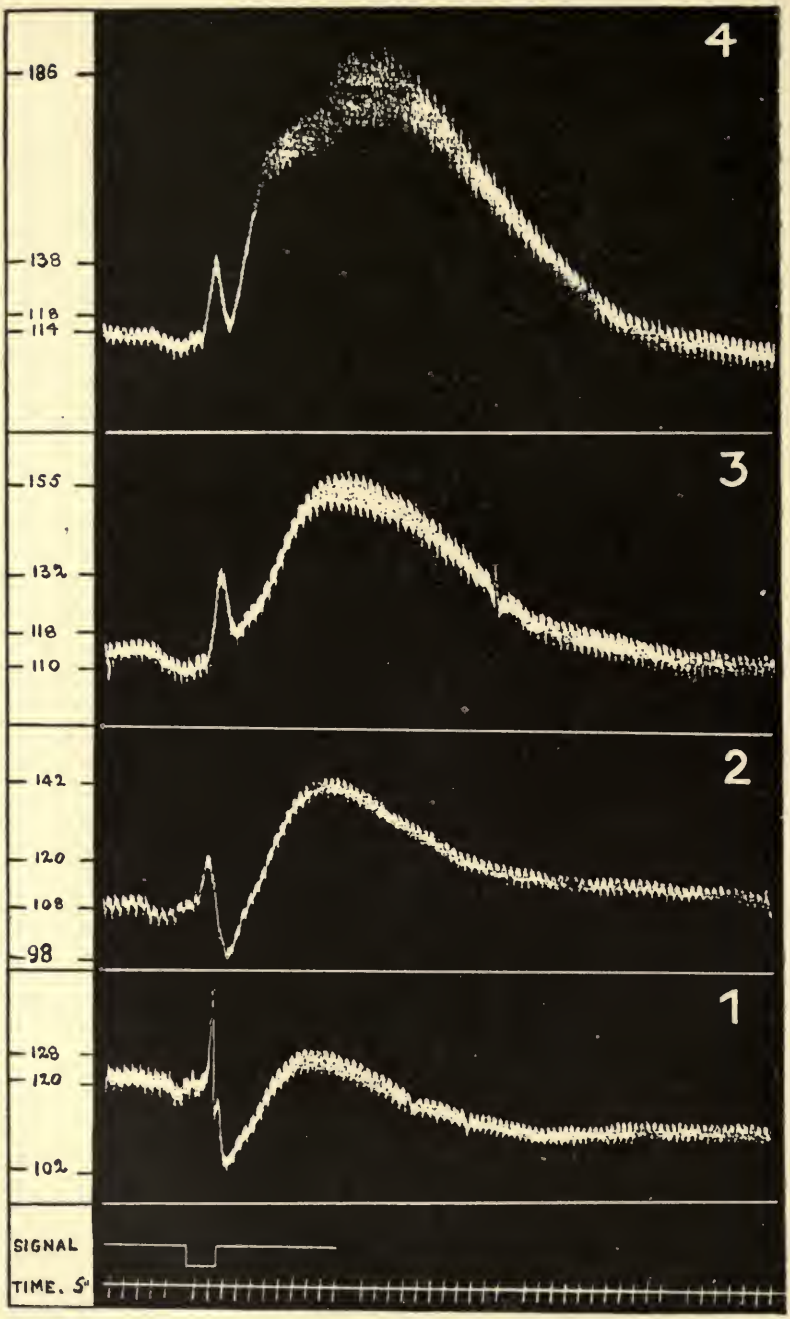


FIG. 51. COMPARISON OF THE ADRENALINE CONTENT—TESTED ON SHEEP—OF THE SUPRARENALS OF PIGEONS IN BERIBERI, NORMAL NUTRITION AND INANITION

I, normal pigeons; II, injection of 0.01 mgm. adrenaline into sheep as control. III, inanition; IV beriberi (McCarrison).

an avitaminosis, and how many of the beriberi symptoms must be attributed to a condition of starvation.

The discovery of glucosuria in pigeon beriberi may evidently be connected with some observations made by McCarrison (l.c. 296). He found that in avian beriberi, the suprarenals undergo considerable enlargement and are characterized by a high adrenaline content. Here too, an increased adrenaline content was found in starving pigeons, but not so much as in beriberi pigeons. The adrenals were also somewhat enlarged. In the same publication we find numerous data on temperature, respiration and weight of the individual organs in avian beriberi, which are also of physiological interest.

In concluding this chapter, we wish to point out that in considering the vitamine B requirements, we must take note of the composition of the diet as regards protein, carbohydrate and fat. These requirements are possibly dependent also on the simultaneous presence or absence of other vitamines. If, for example, the quantity of vitamine A is too little, the requirements for vitamine B are perhaps increased. However, there are as yet no available data on this point.

CHAPTER II

The Antirachitic Vitamine—Vitamine A

The history of the discovery of vitamine A has already been discussed, so that we may take up the chemistry of this substance at once. We have noted that for a long time, vitamine A was supposed to be free from nitrogen, but we feel that in the interest of future investigation this question should be left open. We must also leave for the future, the question as to whether the designation, vitamine A, includes several substances of the same type, or only a single substance. The chemistry of vitamine A has advanced but little so that we shall only discuss its resistance to physical agents, solubility and occurrence.

CHEMISTRY OF COD LIVER OIL

Cod liver oil is the only starting material from which attempts have been made to isolate the vitamine. Although Osborne and Mendel (570) showed in 1914 that this oil contains vitamine A, and although, because of its relationship to the therapy of rickets, it has commanded attention for a long time, we are still in the dark as to the nature of the vitamine contained therein. However, when we consider the known facts about the resistance of vitamine A from various sources, and the technical preparation of cod liver oil (571), it is obvious that because of the stability of the vitamine, cod liver oil is the best material to use for the chemical isolation of vitamine A. Iscovesco (572) believed that almost all of the phosphorus and nitrogen of cod liver oil occurs as a lecithide or as a lecithalbumen. If acetone is added to cod liver oil, with stirring, a precipitate is formed, which, on reaching a maximum, is redissolved. The oil, freed from some of its lecithin by acetone, was shaken with an equal volume of 95 per cent alcohol. To the alcoholic solution, which contains all of the phosphorus of the oil, absolute alcohol was added, giving two separate fractions. The soluble part was concentrated at a low temperature, the residue dissolved in ether and precipitated with acetone. In this way, from 1 kilo of oil, 0.02 gram of the pure lecithide was obtained, which was soluble in benzol and chloroform,

melted at 70°C., and contained 60 per cent C, 7.3 per cent H, 2.1 per cent N and 4 per cent P. A part of this semi-solid mass was used in an experiment on rabbits kept on a normal diet. The lecithide in olive oil was injected subcutaneously every second day for 130 days. The result were as follows:

	Gain in weight per cent
Controls	33
Cod liver oil	55
Cod liver oil without lecithide	37
Olive oil	33
Olive oil with 0.5 per cent lecithide	56

It would appear from these results that the total active substance was present in the lecithide, but it is still necessary to corroborate these findings in rats fed on a diet free from vitamine A.

Other investigators turned their attention to the nitrogenous portion of the oil. In 1888, Gautier and Mourgues (573) showed that even a purified oil contains a small amount of organic bases which at that time were thought to be ptomaines or alkaloids.

One hundred kilos of oil were treated with an equal volume of 33 per cent alcohol, to which 4 grams oxalic acid per liter were added. The watery-alcoholic solution was saturated with calcium hydroxide, filtered and the filtrate evaporated in vacuum at 45°C. At the end of the distillation, calcium carbonate and hydrate were added, the mixture evaporated to dryness aud the residue extracted with 90 per cent alcohol. To this extract, after the removal of the alcohol in vacuum, concentrated potassium hydroxide was added and the alkaline solution extracted with ether. An ether solution of oxalic acid was then added, to precipitate the bases present as oxalates. The yield was 52 to 65 grams oxalate from 100 kilos of oil. The oxalates were dissolved in dilute potassium hydroxide, and the bases set free in the form of an oil, which was dried and fractionated, giving the following fractions:

Fraction I, 87–90°C. butylamine; fraction II, 96–98°C. amylamine; fraction III, under 100°C. hexylamine; fraction IV, between 198–200°C. hydrotoluidine. The distillation was then continued to 215°C. whereupon the brownish distillation residue was extracted with ether. The ether solution was evaporated, the residue treated with HCl and precipitated with a solution of platinic chloride, giving a base as a double salt, called "aselin," having the composition $C_{25}H_{32}N_4$. From the mother liquor, a second base was isolated, called "morrhuin," having the formula $C_{19}H_{27}N_3$. It is not impossible that both of these bases were rather condensation or decomposition products, arising secondarily during the distillation.

This work, which is of little more than historical interest, shows nevertheless that cod liver oil contains nitrogenous substances which are of interest to us because of the vitamine A.

The author (574) has investigated a crude oil and found that the quantity of extractives therein was much greater than in the purified oil. [Crude oil was found more active than refined oil in animal experiments by Zilva and Miura (574a)]. 23.5 kilos of the oil were extracted three times with about 660 cc. alcohol, 2 liters of water and 50 cc. concentrated HCl; the extracts were concentrated in vacuum, the oily residue hydrolyzed with sulphuric acid and precipitated with phosphotungstic acid. The practically dry precipitate weighed 969 grams. The oil, hydrolyzed with sulphuric acid (2 hours) gave an additional 186 grams of this precipitate, while the oil remaining after the concentration of the water-alcohol solution gave only 19.5 grams of the precipitate. A second portion of oil, weighing 25 kilos and treated with sulphuric instead of hydrochloric acid, gave 1117 grams phosphotungstate, of which 61.2 grams was insoluble in acetone. This fraction, after the decomposition with lead acetate, gave a precipitate with sublimate in alcoholic solution. A great number of fractions were prepared by us, but could not be tested for various reasons.

One of the common conceptions regarding cod liver oil was that the mysterious action of unsaturated oils was ascribed to it. However, this view has not yet been experimentally tested. Paal and Roth (575) reduced cod liver oil with colloidal palladium and hydrogen to a solid, whereby the reduction of all the unsaturated bonds was almost completely quantitative. The general nutritive value of such an oil was investigated by Suzuki and his co-workers (l.c. 323) and found favorable. Chapman (576) and a number of Japanese workers including Tsujimoto (577) and Kubota (578) isolated from cod liver oil a very unsaturated hydrocarbon called, "spinacen" or "squalen," which, however, was found valueless as a substitute for vitamine A by Suzuki. Bull (579) obtained a mixture of $C_{20}H_{30}Br_{10}O_2$ and $C_{20}H_{28}Br_{12}O_2$ on brominating cod liver oil. For use in the therapy of leprosy, such products as sodium morrhuate were prepared by Rogers (580).

CHEMISTRY OF VITAMINE A IN BUTTER AND OTHER SOURCES

The data on the chemistry of this vitamine in butter are very uncertain and not infrequently contradictory. The first experiments on the isolation of vitamine A from butter were made by McCollum and Davis (l.c. 82). They reported that when a solution of butter

in petroleum ether is hydrolyzed with alcoholic potassium hydroxide at ordinary temperature, and the soap solution neutralized, the active substance may be concentrated in olive oil by extracting with the latter. Osborne and Mendel (581) dissolved ox fat and butter in alcohol at 40°C. and allowed it to crystallize out on freezing at −20°C. The A-vitamine could not be demonstrated in the crystallized portion, but was found in the oily, uncrystallizable portion. Drummond (582) attempted to repeat the work of McCollum and Davis on the stability after saponification. After butter had been saponified at the usual temperature, the solvent was removed in vacuum, the soaps dissolved in water and the unsaponified portion extracted with ether. All of the fractions thus obtained were tested on rats and found inactive. Recently, Steenbock, Sell and Buell (582a) repeated the saponification experiments and have found that cod liver oil can be treated with 20 per cent alcoholic potassium hydroxide solution for 4 hours at 37°C. without losing its potency. Since the active substance after dilution with water can be extracted with ether, it was concluded that vitamine A is neither a fat nor an ester.

Numerous experiments were carried out for the purpose of concentrating this vitamine from various vegetables and grasses by extraction. McCollum, Simmonds and Pitz (583) thought that the vitamine could not be removed by extracting plant tissue with ether, because it is in all probability bound to the protein. Still, Osborne and Mendel (584) were able to do this. They dried spinach and clover in a stream of air at 60°C., and then extracted it with ether, obtaining a very active substance. These results were confirmed by Zilva (585) who obtained an active alcoholic and ether extract from carrots. Steenbock and Boutwell (586) were unable to obtain an active substance by extraction of carrots with ether alone, but could do so with carbon disulphide, chloroform and benzol. In spite of this, the residues showed almost the original activity. Using alfalfa grass as a source, a simple ether extraction gave no result, while a mixture of alcohol and benzol gave almost quantitative extraction. The same workers then tried to make a fractionation by means of solubility. Alfalfa was treated with cold alcohol, the latter distilled off on the water bath and the residue allowed to stand over night with 20 per cent alcoholic potassium hydroxide. When the saponification had been treated with ether and filtered from the ether-

insoluble portion, the ether solution, after washing with water, was shown to be active. From this residue, the carrotin was separated from xanthophyll by extraction with petroleum ether, showing the carrotin fraction to be active and the xanthophyll fraction, inactive.

THE NATURE OF VITAMINE A

Drummond (l.c. 582) in one of his papers proposed the conception that vitamine A was of the nature of a ferment and was a more or less unobtainable body. He carried out experiments with various known substances and found that fatty acids, glycerol, cholesterol, lecithin, sphingosine, phrenosine, kephalin, lipochrome and carrotin could not simulate the action of vitamine A. Steenbock (587) made the interesting observation that the vitamine A content of maize goes parallel with the content of yellow pigment; white maize was found free from this vitamine, and the same was also true of most kinds of vegetables. According to Steenbock and Boutwell (588) when white corn is supplemented by butter, clover or spinach, the rat resumes growth. Palmer (589), on the contrary, was opposed to this finding. In the first place, he showed that chickens could live on a diet free from carrotinoids, at least through one generation, although it is of course quite possible that chickens may live without vitamine A, or at best with very little. Second, it was possible for him to raise mammals (rats; Palmer and Kennedy, 589a), the blood and subcutaneous tissue of which were pigment free. Despite the fact that the milk of these animals was practically pigment-free, it was possible for them to suckle and rear their young. Palmer observed also that there are oils, such as cottonseed oil, which, although containing pigment, show a lack of vitamine A. Rosenheim and Drummond (590) took up this question and in general were able to confirm the fact that, as a rule, foodstuffs containing lipochrome also contain vitamine A. Still, it was found that a pure carrotin solution is free from this vitamine, and the authors believed that although the latter must be different from carrotin and xanthophyll, it is nevertheless associated with these pigments. As to what action chemically pure carrotin has on rats, opinions differ. While Stephenson (590a) finds that pure carrotin (m.p. 172–173°C.) is entirely inactive and while butter decolorized with charcoal and free from pigment is still active, Steenbock, Sell, Nelson and Buell (590b) have obtained normal growth with pure

carrotin. They prepared vitamine A from alfalfa and found that it withstood saponification with heat, oxidation with hydrogen peroxide and reduction with nascent hydrogen. From the unsaponifiable fraction, active crystalline acetylated products have been prepared. Liver tissue contains, besides the two known yellow pigments, another substance which gives the lipochrome reaction and, in addition, the color reaction with sulphuric acid which is regarded in the literature as being specific for cod liver oil. This substance is not a casual impurity as it may always be demonstrated in cod liver oil and in liver fats. Hijmans van den Bergh and Muller (591) have described methods which may be used for the separation of the lipochromes in blood. In order to liberate them, it was necessary to precipitate the serum proteins with alcohol. It was assumed that other chemical substances were associated with the pigments. Drummond and Coward (592) have investigated the pigment and vitamine A content of a number of oils and fats, and found that these two factors do not always occur together. In butter, the pigment content was found to run somewhat parallel to that of vitamine A, whereas certain pork fats and dog fat contain no pigment, but a marked amount of vitamine. They came to the conclusion that a relationship between the pigment and vitamine A could not be maintained unless we assume, with Steenbock, that in some cases the pigments occur in the leuco-form. As evidenced by the above, the whole question still rests on an uncertain foundation and can be solved only when a purification of this substance is made.

Properties of vitamine A. The characteristics of this vitamine are as yet very little known. As for the question whether the substance is soluble in water, it must remain open for the present. McCollum, Simmonds and Steenbock (593) have stated that fat-free milk still contains appreciable amounts of vitamine A, a finding which seems to be in accord with the latest results. According to their work, the vitamine may be extracted with small quantities of water from melted butter by repeated extraction, the remaining butter being inactive. On the other hand, Steenbock, Boutwell and Kent (594) showed that both the residual butter and the watery extract were inactive. They believed that heat and not oxidation is the reason for the destruction, although we shall see that there is still another complication to be considered. The finding of McCollum, Simmonds and Steenbock (l.c. 593) that fat-free skimmed milk still contains vitamine A was again confirmed by Hopkins (595).

The first statement on the stability of the active substance from butter was made by Osborne and Mendel (l.c. 581). They found that when steam was passed through butter for 2½ hours, the vitamine was unaffected. That this substance in egg-yolk is resistant to heat, was shown by McCollum and Davis (596). In this connection, Drummond (597) found that vitamine A from butter and other sources is very unstable to heat, thus corroborating the statements of Steenbock. Drummond also reported that oxidation does not seem to play any part in the destruction of this substance. Steenbock and Boutwell (598) found yellow corn and alfalfa active after heating in the autoclave for three hours. Osborne and Mendel (599) found butter, as such, much more stable against storage and the influence of light, than the uncrystallizable part—butter oil.

All of these apparently conflicting statements may be well understood when the new observation of Hopkins (l.c. 595 and 599a) is taken note of. He found that in the destruction of vitamine A oxidation, not temperature, plays the chief part. This has been confirmed by Drummond and Coward (599b) and is in agreement with the observation of Osborne and Mendel (600), who found butter stable when steam was passed through it, but not when the butter was heated in the absence of water. The stability in this case may be explained by the replacement of air by steam, although even dry butter could be heated at 96°C. for 15 hours without noting any loss of activity.

As regards the action of physical factors, Zilva (l.c. 513) showed that butter lost its activity on exposure to ultra-violet rays for 8 hours. The possibility of the formation of ozone was considered in a later paper, and it was actually found that ozone is destructive to vitamine A (600a).

Although the destruction of vitamine A by oxidation has been well explained by various statements, it is more difficult to understand the destruction of fat on reducing at a temperature of 75° to 120°C., as carried out by Fahrion (601). For, in the first place, vitamine A, according to the newer findings, is quite resistant to heat, and second, the substances sensitive to oxidation are, for the most part, resistant to reduction. Nevertheless, according to the findings of Drummond and Coward (l.c. 592), as well as of Fahrion, the reduced fats are totally inactive. Fahrion believed that vitamine A is almost insoluble in fat solvents.

Considering the manner of preparing commercial cod liver oil, we must marvel, in the light of the above mentioned observations, that the oil still has therapeutic properties. In this connection, it would be interesting to observe the action of oxy-cod liver oil—a preparation which, according to Freudenberg and Klocman (602), is made by oxidation of cod liver oil with hydrogen peroxide—as well as cod liver oil emulsions prepared by shaking in alkaline medium. The influence of such a procedure on the therapeutic action is discussed by Hess and Unger (603). If the vitamine A of cod liver oil really withstands the above mentioned treatment and long storage, then we must assume that the vitamine from this source is exceptionally stable, a characteristic which should be significant in further studies. According to Zilva and Miura (l.c. 574a), cod liver oil contains 250 times as much vitamine A as butter, and 3 milligrams of this oil per day is sufficient for rats. Drummond, Coward and Watson (603a), found that certain samples of butter contained no more vitamine A than refined vegetable oils. The above data undoubtedly explain the differences observed in the value between cod liver oil and butter in the treatment of rickets.

DEMONSTRATION OF VITAMINE A

Till now, the only method used, as we have already seen, is the behavior of the rat. To make the demonstration definite, it is necessary, according to Drummond and Coward (604) to provide, above all, that the basal diet is completely free from this substance. The basal diet used for this purpose is composed as follows:

Purified casein	18	Yeast extract	5
Purified rice starch	52	Orange juice	5
Purified reduced vegetable oil	15	Salt mixture	5

The casein is heated in open dishes for 24 hours at 105°C. Rice starch has been shown to be pure for this purpose and may be used, as such. Completely reduced vegetable oil, usually cotton seed oil, or steam distilled palm-kernel oil (Stammers, 604a) is used as a fat. If one is uncertain as to the freedom of this oil from vitamine A, then the addition of fat should be omitted entirely. Orange juice is free from vitamine A_2, while yeast extract has not yet been investigated sufficiently in this respect. Rats weighing 50 to 70 grams, and 4 to 5 weeks old should show no growth at all, on this

diet. If growth ensues, it may be concluded that the basal diet was insufficiently purified. Older animals, weighing over 100 grams should not be used since the vitamine A requirement diminishes with age. If the growth remains stationary during 10 to 14 days, the substance to be tested may be added, preferably apart from the diet. If growth is seen, it may be concluded that vitamine A was present in the substance added.

Significance of vitamine A in physiology and pathology. From a study of this vitamine in various nuts, Coward and Drummond (605) came to the conclusion that it occurs only in small amounts in seeds, but that during the process of germination it appears in greater quantities. We have already mentioned the possibility that green leaves may synthetize vitamine A under the influence of sunlight, and in this connection Delf (605a) has found that the inner white leaves of cabbage are free from this vitamine, while the outer green leaves contain the substance. Recently, Coward and Drummond (605b) have experimentally substantiated these findings, showing that green leaves form vitamine A only in the presence of sunlight, while the lower organisms devoid of chlorophyll are unable to synthetize this vitamine.

Hughes (605c) fed a number of animals on diets rich and poor in vitamine A, but on examination of the organs, no relation could be shown between the vitamine A content of the food and that of the adipose tissue. Drummond, Golding, Zilva and Coward (605d) explain the poverty of pig's fat in vitamine A in two ways (1) inadequacy of the diet in this substance, and (2) losses by oxidation during the melting out process of the fat.

We have already stated that the lack of vitamine A in rats is followed by no characteristic pathological changes, and hence it is difficult to point out just what is the importance of this substance. Drummond (606) tried to solve this question. Since this substance is associated with fats, as a rule, it was easiest to assume that it played a part in the fat metabolism, but it was not possible to find such a relationship. Rats lacking both this vitamine and fat showed no different behavior from those animals lacking only one of these constituents. The temperature of these rats was normal, so that this substance has nothing to do with the regulation of body temperature. In another experiment, Drummond tried to see whether or not vitamine A plays a rôle in the synthesis of neutral fats from

fatty acids. For this purpose, one series of animals were fed with fat, another with fatty acids. When vitamine A was absent from the diet, the fatty acid mixture was well assimilated, and the animals showed a normal fat content. In our experiments, together with Dubin (l.c. 331), we fed rats on a diet containing a large amount of neutral fat. These rats, in spite of the administration of vitamine B, ceased growing and lost markedly in body weight. We tried to improve the condition of the animals by adding greater amounts of vitamine A in the form of cod liver oil or butter, but without success. Unlike vitamine B, vitamine A, given parenterally, is without any action.

The rôle of vitamine A in pathology was treated by Mendel (607). It was particularly noted that this substance was of less importance for adults than for young growing animals. It was also shown to be of significance in the manifestation of ophthalmia, as well as in the occurrence of urinary calculi, conditions which arise chiefly when vitamine A is absent from the diet. McCarrison (608) holds that vitamine A plays a part in the prophylaxis of edema. All of these statements will be discussed in relation to each other in another chapter.

CHAPTER III

The Relationship of the Antiberiberi and the Antirachitic Vitamines to Lipoids

The object of this chapter is to show that vitamines B and C have presumably not the slightest relation to the lipoids. It is, of course, more difficult to demonstrate this as regards vitamine A. The association of these two classes of substances, the lipoids and the vitamines, came about through the conception that lipoids, and especially lecithin, are not pure products but may contain vitamine as an impurity. It has often been stated by Cronheim (609), and by others, that lecithin is of significance in metabolism, while Robertson (610) noted only an inhibition of growth on feeding this substance to mice.

In biochemistry, the idea has long been prevalent that when animal or plant tissue is extracted with alcohol, or better still, with ether, the fraction in the solvent can be regarded as a lipoid. In our first investigation, carried out in the Lister Institute in 1911, which had for its purpose the isolation of vitamine, we convinced ourselves that an extraction with alcohol removes a great deal of nitrogenous matter (purines, pyrimidines, etc.), in which the vitamines are also to be found. This observation was made use of by McLean (611), working in the same Institute at that time, to remove nitrogenous impurities from lecithin. For this purpose, a crude lipoid from horse meat was finely divided and repeatedly extracted with water. In the first alcoholic extract, as well as in the watery extracts similarly obtained, the presence of carnosine, hypoxanthine and vitamine was demonstrated with certainty. For the purpose of vitamine isolation, these mother liquors were given by McLean to Cooper (l.c. 535) who readily fractioned out the vitamine. For the above mentioned reasons, it is likewise evident that the growth-promoting influence of the lecithides of the liver, isolated by Iscovesco (612), was due to the presence of vitamine. The same is perhaps true for tethelin, isolated from the hypophysis by Robertson (613). This lipoid, regarded as pure, gave reactions for purine bases, as well as a diazo reaction, so that it is certain that we are not dealing with a pure lipoid in this instance.

As regards vitamine B, Cooper (614), and later Sullivan and Voegtlin (615), showed that there is no relationship between the lipoids and vitamine B. As for vitamine A, the behavior is quite different, but still there is no reason for saying with Aron (l.c. 329, 616) and Stepp (617), that lipoids are essential for life. In the first place, with a mixture consisting of the known lipods, lecithin, kephalin, cerebron and cholesterol (Stepp, l.c. 352), only a slight effect could be discerned, due perhaps to the cumulative action of all the impurities. Secondly, Stepp (l.c. 351) showed that at least one type of animal, the pigeon, could live in the presence of vitamine B, without lipoids. Recently, Stepp (618) stated that dogs and rats cannot live on a lipoid-free diet, upon which the animal develops paralysis of the hind extremities, but not beriberi. An addition of vitamine B prolongs the life of the animal but cannot save it from death. This experiment was supposed to show that animals die because of a lack of lipoids. Regarding the matter from the viewpoint of the relationship of vitamine A to the lipoids, we see that while at first the view was held that this vitamine was connected exclusively with the animal fats, it was found later that green leaves likewise contain large amounts of the same substance. Vitamine A is supposed to be present in skimmed milk, too, and since the latter is believed to be free from lipoids, the relationship between vitamine A and lipoids must necessarily be non-existent. In particular the question as to the solubility of vitamine A in water has not yet been cleared up; besides, in the extraction of a fat with water, as we have already observed, the possible oxidation is to be taken into account. Myers and Voegtlin (l.c. 503) shook out the vitamine of yeast with olive oil, and showed that besides the vitamine, other nitrogenous substances are taken up in the oil. In this case, we must regard the total oil soluble portion as lipoid or, at any rate, as fat-soluble. Since it has never been shown that vitamine A is a constituent of a pure lipoid, and since up to the present all purely isolated lipoids have given negative results when tested for their activity, there is no reason for considering vitamine A as being associated with lipoids; it may ultimately be shown that this association is only incidental. Cramer (619) described a new type of glandular fatty tissue, which he called "lipoid gland" or "cholesterol gland." This tissue, which is very rich in cholesterol and other lipoids, loses these constituents when the animal is placed on a

vitamine-free diet. Cramer believed that this tissue plays a part in the development of avitaminoses, in that it functions as a vitamine reserve of the body. The exact functions of this new fatty tissue are not yet known, but Cramer thinks that a close relationship exists between this tissue, the thyroid and the suprarenals.

CHAPTER IV

The Antiscorbutic Vitamine—Vitamine C

THE CHEMISTRY AND THE NATURE OF VITAMINE C

We owe to Holst and Frölich (l.c. 359) our first knowledge on this subject. They showed that fresh potatoes, cabbage, dandelion, carrots, raspberry juice, lemon and sour dock juice contain this vitamine. They observed the interesting fact that the various antiscorbutics differ markedly in the stability of the vitamine present, evident on heating, storage and drying. As to the reason for this, we are still in the dark. We do not know if in these cases we are dealing with different or with the same substances, and yet the varied behavior towards external factors is not necessarily due to the presence of distinct substances. The different combinations of the vitamine and the characteristics of the juice may be responsible for this behavior.

It may be said in general that antiscorbutics exhibit less activity when boiled than when in the natural condition; furthermore, heating to 110° or 120°C. is harmful, even more so than boiling at 100°C. It is not only the heating that exerts a harmful effect, but also the incidental drying, which is in accord with the experience gained in the study of human scurvy. According to Holst and Frölich, the relative moisture of the drying chamber plays a part, since the antiscorbutic activity of potatoes, carrots, dandelion and cabbage is more quickly lost at room temperature than in an incubator at 37°C. The dried dandelion, unlike cabbage, is completely inactivated. Vegetable juices behave differently; for example, cabbage juice loses its activity when heated for 10 minutes at 60°, 70° or 100°C.; similarly, when it is stored at ordinary temperatures (in the presence of preservatives) or in the ice-box. In the next chapter we shall speak of the effect of the cooking on the antiscorbutic properties of various foodstuffs.

Lemon juice behaves quite differently from the above mentioned substances, as does also milk. The former may be heated to 110°C. for 1 hour without any noticeable loss of activity. Now this juice contains 7 per cent citric acid, and Holst and Frölich held that

vitamine C was more stable because of the acid present. This idea was confirmed by the fact that other acid juices, like raspberry and sour dock juice, show the same stability towards heating. The heat stability of cabbage and dandelion juice was markedly increased on the addition of acid, but permanent stability could not be obtained.

The last finding was made use of in extractions. If freshly dried cabbage is extracted with alcohol to which a little citric acid is added, instead of with pure alcohol, the extracts thus obtained are much more active on guinea pigs. Other isolation experiments, such as dialysis or extraction with petroleum ether, were valueless. Holst and Frölich were unable to favorably influence scurvy in guinea pigs by intraperitoneal injections.

In a later communication, Holst and Frölich (620) stated that cabbage dried at 37°C. and stored in a desiccator still contained vitamine C after 13 months. It was possible also to obtain an active substance by extracting cabbage with a mixture of alcohol and glycerine. This was also the case with extractions made with dilute alcohol, to which 0.5 per cent citric acid had been added. Hot 80 per cent alcohol with 0.5 per cent citric acid likewise extracts vitamine C from freshly dried cabbage. Since the experiments of Holst and Frölich in 1913, no real progress has been made, in spite of the numerous publications that have appeared.

Shortly thereafter, the author undertook a chemical fractionation of vitamine C, using the same methods as in the yeast fractionation, but the results obtained were not very satisfactory. The investigation failed chiefly because of the lack of knowledge of scurvy in guinea pigs, as well as of the behavior of vitamine C which at that time had hardly been recognized. Although more firmly established data are available on this subject at present, the chemistry of this vitamine has thus far not progressed. In 1912 we investigated, in this respect, milk, potatoes and lime juice, using guinea pigs and rabbits kept on water and oats. The guinea pigs died in about 20 to 30 days with marked scorbutic symptoms. After the addition of fresh potato juice, the animals died after 36 days, while after the purification of this juice with lead acetate, the animals died after 27 days. A daily addition of 25 cc. milk had a definite inhibitory effect on the scorbutic symptoms, while on addition of 50 cc. milk, the symptoms of scurvy were absent even after 50 days, the animals sometimes gaining weight; we shall discuss the dissimilar results of

Chick and Hume in the chapter on milk. Protein-free milk, prepared by precipitation of casein with acetic acid at 50°C., lactalbumin removed by colloidal iron solution, and concentrated by freezing (this may perhaps be a practical method of concentrating vitamine C), exhibited, in amounts corresponding to 200 cc. of fresh milk, only a very slight activity. An alcoholic extract of milk dried in vacuum by us, possessed no activity in large doses, while the residue, given in doses of 30 grams showed definite activity in some cases. We then turned our attention to lime juice (l.c. 101), which was universally known as a splendid antiscorbutic. For this reason we were unable at that time, to explain why it was inactive in guinea pigs. The same result was also obtained with the decomposed phosphotungstate, prepared from lime juice. We were beginning to doubt the identity of scurvy in guinea pigs with that in man, though recently the situation was completely explained by Alice Henderson Smith (621). In a historical sketch, she showed that the statements as to the protective action of lime juice on scurvy were not true for the sour limes of the West Indies used to-day, but were so for sweet limes and lemons growing in the Mediterranean regions.

The lime juice used to-day was shown to be inactive, but we demonstrated the presence therein of vitamine B. Aside from this, we isolated a terpene having the formula $C_{13}H_{24}O_3$, a purine base, $C_6H_7O_2N_5$ (m.p. 282°C.) and a substance from the histidine fraction, melting at 188° to 189°C. and having the formula $C_9H_{18}O_6N_2$. From the choline fraction, a base was isolated as the double platinum salt, which melted at 220°C. and which was given the formula, $C_8H_{15}O_2N$, though a later investigation showed that the substance was identical with stachydrine $C_7H_{13}O_2N$. These data are quoted here because it may later be shown that one of these substances is a cleavage product of vitamine C.

The experiments of Holst and Frölich on the extractability of vitamine C with alcohol, were later confirmed by Freise (622) on turnips, as well as by Freudenberg (623). Subsequently, Harden and Zilva (624) showed that when orange juice is freed from citric acid by precipitation with calcium carbonate, and the mixture treated with an equal volume of alcohol, filtered, and the filtrate concentrated in vacuum at 37°C., an active solution is obtained which, however, loses its activity in time. If lemon juice is treated similarly, and concentrated in acid reaction, an active residue is also obtained.

Later, Harden and Robison (625) showed that vitamine C is not volatile on distillation, that the dry residue has almost the same activity as the original juice, and that it may be kept in a dry container for six months. Apple juices were found to be much poorer in vitamine C. This applies to juices concentrated in the laboratory; when prepared in large amounts, these preparations lose 63 per cent of their activity, according to Harden and Robison (626). Vedder (626a) prepared active extracts by successive extractions with absolute alcohol acetone and ethyl acetate. Vitamine C was destroyed by phosphotungstic acid and the last extract exhibited about one-seventh of the initial activity and contained only traces of nitrogen. This led Vedder to the somewhat premature conclusion that vitamine C is nitrogen-free. Without going further, we see that active preparations of vitamine C may be obtained also in the absence of citric acid, and we are therefore not yet certain whether these acids possess protective qualities.

Givens and McClugage (627) dried orange juice in two different ways. First, in shallow dishes at 55° to 60°C. for 50 hours; second, according to a method used in drying milk (Just-Hatmaker process), in which a quick drying at 75° to 80°C. is obtained. Whereas the second preparation was almost as active as the original juice, the first lost a great part of its effectiveness. The active products were kept for $3\frac{1}{2}$ months without loss of activity. Stable preparations of vitamine C were made by Dubin and Lewi (628) and also by Bassett-Smith (629). As regards the stability of vitamine C to alkalis, a number of papers have been published. Harden and Zilva (630) made orange juice alkaline with a $\frac{N}{50}$ sodium hydroxide and kept this for 24 hours, resulting in a loss of activity. Hess and Unger (631) carried out similar experiments and believed that, for the most part, storage in alkaline medium exerted an unfavorable influence on the activity. Accordingly, if the juice is boiled up in a slightly alkaline solution and immediately neutralized, the activity is only slightly diminished. McClendon and Sharp (632) investigated the reaction of natural juices to determine if they are not somewhat alkaline, which would explain the destruction of vitamine C on boiling; however, most of the juices were found slightly acid.

Sommer and Hart (633) stated that the destructive action of boiling on vitamine C was not due to the precipitation of citrates, while Faber (634) found that an addition of sodium citrate (even in a concentration of 0.25 per cent) has a destructive action on this vitamine.

As regards the oxidizability of vitamine C, Fowler (635) showed that milk preserved with hydrogen peroxide is scurvy producing. Hess (636) believed that milk or canned tomatoes shaken with air lose considerably in their antiscorbutic activity.

According to Zilva (l.c. 513) ultra-violet light has no influence on the activity of vitamine C. Adsorption likewise does not affect this vitamine. It may be treated with fullers earth or colloidal iron, according to Harden and Zilva (l.c. 89), and it may also be filtered through a Berkefeld filter without loss of activity. Zilva and Miura (636a) were unable to separate vitamines B and C by means of differential dialysis.

As for the significance of vitamine C in the organism, very little is known, and what little knowledge there is available will be described under human scurvy. The question as to whether the parenteral administration of vitamine C is effective has been answered differently by different investigators. Holst and Frölich (l.c. 359) gave this vitamine intraperitoneally, and Harden and Zilva (l.c. 624) found it inactive on subcutaneous injection. Hess and Unger (637), on the contrary, working with children, showed that intravenous therapy with weakly alkaline orange juice is possible, and they recommend this procedure.

Here, too, as with vitamine B, Hess (638) accepted first that a large addition of carbohydrates hastens the onset of scurvy, but he (639) has changed his view recently. Hess has tried by addition of 3 per cent flour to the milk to hasten the onset of scurvy in children, with negative results. We do not believe, however, that this amount of carbohydrate is sufficient to influence the effect one way or the other.

CHAPTER V

Vitamine Content of Various Foodstuffs, in the Natural and Prepared Conditions

We have already shown that during the last few years most foodstuffs have been investigated for their nutritive value and vitamine content. The results obtained give us an insight into the comparative values of the above, and yet we must be careful in interpreting the data. First of all, it should not be forgotten, as frequently happens nowadays, that the results obtained with one animal species cannot, ipso facto, be applied to others.

For instance, it seems to us unjustifiable to determine the nutritive value of an animal diet by using animals that normally live on plant diet; this may be the cause of the wide differences between experiments on man and those on animals. Variations in the antiscorbutic value of meat in man and guinea pigs may be due to just such procedures.

Another difficulty in the interpretation of results is the lack of quantitative methods for the estimation of the vitamine; this is most apparent in the comparison of the results obtained at the outset of vitamine research, with those of modern investigations carried out with a better understanding of this new subject. Many of the older results were obtained without paying any attention to quantitative relationships, and Chick and Hume (640) correctly noted that in this regard, we should be constrained to work quantitatively. For example, if we would wish to study the effect of alkali on vitamines, it does not suffice merely to add alkali to the foodstuff and feed it to the animal. We must first determine the minimal protective dose of the natural preparation, for if too great a dose is used from the start, the preparation, in spite of an extensive destruction, may still contain sufficient vitamine to act therapeutically, and we would make the erroneous conclusion that the treatment to which the foodstuffs had been subjected was without influence on the vitamine content.

Most of the animal experiments conducted up to the present suffered because of the fact that with the addition of vitamine in the form of food, or in the form of extracts, the composition of the basal diet was at the same time altered, without having the corresponding

controls with no vitamine addition. Most of the investigators also regarded the vitamine content of any particular diet as an unchangeable constant, as a mathematical certainty. We shall see in this chapter that this is not at all the case, and it will become clear how the various investigators could have obtained dissimilar results with the same food.

After pointing out the possible relationship between vitamines and appetite (Mendel 641), and the necessity of new legislation for foodstuffs with regard to the vitamines (Ranwez 642), we shall take up the description of the treatment of foodstuffs as related to the vitamine content. Here, there are the effect of heating, cooking, drying, storing, sterilizing, and preserving. Here, too, we shall describe the more important foodstuffs commonly used in human nutrition. It will be impossible, however, to go into all of the investigations made in this respect, and for this reason we shall put all of the data considered of importance in the form of a table at the end of this chapter, showing the relative richness of the foodstuffs in the three known vitamines. The first chart of this kind was made by Cooper (643) in 1912-14, including but a few foodstuffs and concerning itself only with the vitamine B content. A similar table was given in the Report of the Medical Research Committee (l.c. 333) wherein it was attempted to record quantitatively the values found. The values for vitamine B are recorded, compared to 100 for wheat germ.

FOODSTUFFS	VALUE	MOISTURE CONTENT
		per cent
Wheat germ	100	10–13
Wheat bran	25	10–13
Rice germ	200	10–13
Pressed yeast	60	70
Dried peas	40	12
Lentils	80	
Egg yolk	50	70
Ox liver	50	70
Ox muscle	11	75
Potatoes	4.3	80

In the same report, we see also the first investigation of the content of other vitamines. All of these values were obtained on animals and apply strictly only to the species upon which the results

were determined. This is true especially as regards quantitative relationships. Chick and Dalyell (644) prepared another table with regard to vitamine C, where the values are based on 100 for lemon juice.

Fresh orange juice or cabbage leaves	110
Fresh raw orange juice	100
Beet juice (swede)	60
Green beans	30
Sprouted fresh peas	30
Carrot juice	7.5
Red beet juice	7.5
Meat juice (ox)	7.5
Potato (cooked 39 minutes)	7.5
Fresh cow's milk	1–1.5

In our table, we were not satisfied to give only the vitamine value, but also the nutritive value of the proteins of various origin. This rubric will, perhaps, help us to demonstrate the difference between the nutritive value of plant and animal protein, which may be of importance in relation to the etiology of pellagra and war edema; unfortunately, however, the data found on this subject are very meagre. It appears to us not impossible that some protein substances may contain adsorbed either a fourth vitamine, or a hitherto unknown amino acid, essential to life.

INFLUENCE OF HEATING AND COOKING ON THE VITAMINE CONTENT[1]

We owe to Schüffner and Kuenen (l.c. 56) the first data on this subject of such practical importance. Studies on the stability of vitamine B in foodstuffs were undertaken also by Grijns (l.c. 52). Recently this field was systematically investigated by Chick and Hume (645), using an improved technique. They studied the influence of heat on vitamine B of wheat germ and found that a temperature of 100°C. for two hours has little influence, while a temperature over 120°C. is quickly followed by destruction. Miller (646) found that cooking and autoclaving (at 115°C. for 45 minutes) had no effect on carrots and beans; this was confirmed by Whipple (647) for cabbage and onions. In the last two investigations the content of vitamine B was determined by the yeast method already described

[1] A good review of the known data is found in the paper by Emmett and Luros (l.c. 94).

by us, but was not controlled by animal experiments. The stability of vitamine C against heat depends very much on the nature of its source. Weill and Mouriquand (648) stated that this vitamine is very labile, but noted that lemon juice may be heated to 110°C. for 1 hour without marked loss in activity. Delf and Tozer (649), coworkers of Chick, carried out investigations on cabbage: heating for 1 hour at 60°C. decreased the antiscorbutic value about 70 per cent, at 90°C, about 90 per cent, while on short heating (20 minutes), at 90° or 100°C., the activity is decreased only about 70 per cent; we see from this that the chief influence is produced by the time of heating and not by the temperature. Strudwick (650) investigated, in this respect, the influence of cooking in a fireless cooker in which the time of cooking was markedly prolonged. The results showed that a rapid heating is much superior to the above method.

Delf (651)[2] investigated, in the same way, the relationship between cabbage leaves and cabbage juice, showing that in the natural condition, vitamine C is much more resistant. Turnip juice (swede) was shown to be far more stable than cabbage juice. Orange juice heated to 130°C. lost 50 per cent of its activity. Givens and McCluggage (652) found tomatoes quite resistant to heat. However, if they are heated for 15 minutes or more at 100°C, a greater amount, in comparison with the crude material, must be fed in order to protect guinea pigs against scurvy. Aside from heating, oxidation apparently also plays a rôle in the inactivation of vitamine C. Rossi (653) fed guinea pigs on oats and hay sterilized in open kettles at 126°C., with the result that the animals died of scurvy. However, a different result was obtained when the food was heated as above in closed kettles; on this food, the guinea pigs were still alive after two months. Delf (l.c. 651) also believed that in the presence of air the inactivation of vitamine C was hastened, and this condition, according to Steenbock, Boutwell and Kent (l.c. 594), and others, plays a part in the destruction of vitamine A.

The destruction of vitamines by heat depends on still another factor—the chemical reaction of the starting material. In this connection, the data are not so certain for vitamine B. While McCollum and Simmonds (l.c. 315) reported on the supposed destruction

[2] In comparing the temperature coefficient of the heat destruction of vitamine C with toxins and ferments, Delf concluded that this vitamine must possess a relatively simple chemical structure.

of this vitamine by alkalis, Daniels and McClurg (654) stated that they observed no destruction of cabbage and soya beans so treated. It is possible, however, that the latter investigators fed too much of the active material. According to Daniels and Heisig (655), the effect of the addition of soda upon vitamine C was deleterious. In this connection, Hess and Unger (656) point out that soda is used in the preparation of proprietary infant foods. This is true also of commercial cod liver oil emulsions, which give an alkaline reaction, and to which soda is added in order to obtain a better emulsion. Yet we have no exact experiments to show whether the activity of the above emulsions is affected by the manner of preparation. Experiments started by us have not yielded any definite results up to the present. We must say, however, that the vitamine A of cod liver oil must be quite resistant in order to withstand the treatment undergone during the process of manufacture.

It has often been stated that the stability of orange juice is explained by the acid reaction of the medium. Still, according to the findings of Delf (l.c. 651), the neutralization of the juice did not have any harmful effect.

Summing up the subject matter of this important chapter, we may say that the stability of the vitamines on heating depends not on the temperature but on the duration of the heating. In this respect, the view of Givens and McClugage (657) is of interest; they believed that when antiscorbutics are heated at a low temperature for a long time, as in gradual drying, the ferments contained in the tissues destroy the vitamine. If the ferments are inactivated by rapid heating at high temperatures, then vitamine C is much more resistant. These statements naturally require further corroboration.[3] Aside from this, the vitamines seem to be more stable in the natural condition than in the form of extracts or pressed-out juices. Oxidation plays a big part in the destruction of vitamines A and C, while the chemical reaction of the substrate plays a part in the destruction of all the vitamines.

[3] This behavior may be brought into relationship with the stability in acid solution, if it is assumed that these ferments act in alkaline solution; we have in mind here the oxidases, the action of which is delayed in acid medium.

The vitamine content of cooking water

That vitamines may be lost by discarding the cooking water, was first stated by Schüffner and Kuenen (l.c. 56). In addition, vitamines are lost in other water-soluble substances and it is therefore not without reason that Hindhede (658) pointed out the great nutritive value of soups, especially vegetable soups. Hill (659) stated, for example, that on cooking potatoes, losses ensued which represented 70 per cent of all water-soluble substances. From the viewpoint of vitamines, the same thing was pointed out by a number of workers—Denton (660), Daniels and McClurg (l.c. 654), Miller (l.c. 646) and Whipple (l.c. 647). The last two investigators, in particular, found that by discarding the cooking water, 30 to 70 per cent of the substance necessary for the growth of yeast was lost.

THE INFLUENCE OF DRYING

Since the question of the dehydration of foodstuffs has already assumed practical importance, it is regrettable that comparatively little is known of its effect on vitamines. Murlin (661) believed that the nutritive value of dried foodstuffs is strictly comparable with that of fresh. Prescott (662) put the practical side of this problem to the fore, in relation to transportation and storage, particularly for the provisioning of any army in the field. He stated also that the loss of vitamine C on drying may be prevented by modifying the procedure. Givens and Cohen (663) investigated this question, using cabbage and potatoes. Drying in a stream of air at 40° to 52°C. resulted in an appreciable loss of activity. With the simple drying, the preparation of foods is not yet completed, and practically two other factors are to be considered—the storing and the cooking just before eating. These two factors have unfortunately not been considered by most investigators; nevertheless, it was found that when cabbage or potatoes were first heated or cooked and then dried, no more vitamine C was left, as shown in guinea pig experiments.[4]

[4] Holst and Frölich (663a) found that if moisture is excluded as much as possible to diminish the chances of hydrolysis, a stable cabbage preparation, as regards vitamine C content, may be obtained. The dehydration was carried out in vacuum at 37°C. in presence of phosphorus pentoxide, which is replaced as soon as it becomes moist. This preparation was kept for 10, 18, and 26 months and found active.

Givens and McClugage (664) found tomatoes more resistant in this respect, at least in relation to storage after drying, since after three months, the activitv was still retained. Subsequent cooking experiments appear to have not been made, but are not particularly important inasmuch as tomatoes may also be used raw. Shorten and Ray (665) dried various kinds of vegetables in the sun, and investigated their vitamine B and C content. The results showed that vitamine C of carrots, onions, and cabbage were still present in sufficient amounts for guinea pigs. Spinach, on the contrary, lost this property. We find no statements here to the effect of ageing and subsequent cooking. Falk, McGuire and Blount (666) studied the ferments of dehydrated vegetables. They found them inactivated and pointed out the similarity to the resistance of the vitamines. Givens and McClugage (l.c. 657) published a very careful investigation upon the drying of potatoes. Taking their work as a whole, we find that when potatoes undergo a series of treatments, the individual effects are summed up, resulting in a totally inactive product. If the potatoes are boiled, steamed or baked and then dried, an inactive product is obtained, as a rule. Drying alone does not have this destructive effect. The vitamine was least affected when the potato was baked in the jacket (perhaps on account of absence of air) and then dried. Addition of acids before treatment did not have the desired effect. Potatoes dried in vacuum also lost considerable activity. We have already seen that quick drying at high temperatures is less destructive than gradual drying at low temperatures. Ageing and subsequent drying were not investigated, although it is clear from what has already been said that vitamine C of potatoes is not resistant.

CANNING

This subject too has not yet been extensively investigated. We believe that for this question, the foodstuffs may be classified in two groups—those used raw, and those to be cooked before consumption. We find the conditions here similar to those in drying. Bigelow (667) described the problems arising in canning. Different sources of supply give rise to different conditions so that general conclusions can hardly be drawn as yet. Hess and Unger (668) found that canned tomatoes still showed a splendid antiscorbutic action in children, and adopted this antiscorbutic in their children practice; it is specially suitable for institutions since the expensive orange juice may be

substituted by something cheaper. This finding is of practical significance, since in the anticipation of scurvy epidemics, canned tomatoes, which appear to keep well, may be used, though it should be remembered (Hess and Unger, l.c. 631) that the activity decreases on cooking. Daniels and McClurg (l.c. 654) believe that the canning proces does not destroy vitamine B; it seems to us that an absolute proof of this has not been obtained in canning, although they admit that changes initiated during canning may progress automatically during storage. Campbell and Chick (669) prepared cabbage and beans in a manner similar to that used in canning, and found that these substances lost about 66 per cent and 75 to 90 per cent of their activity, respectively. Vitamines A and B were also affected although the investigators explained it by losses of vitamine A into the cooking water. When this was given to guinea pigs, it resulted in better growth. We must assume, therefore, with Campbell and Chick, that vitamine A went over into the cooking water.

AGEING AND STORING

The last factors to be taken up in this connection are those of ageing and storage. Hulshoff Pol (670) could cure human beriberi with purée of peas, although peas, which were three years old, were entirely inactive. Weill, Mouriquand and Michel (l.c. 392) found the same to be true of denatured meat, stored for 14 months. For cats, this meat was much more harmful than freshly denatured meat. Delf and Skelton (671) dried cabbage and stored it at ordinary temperature; after 2 to 3 weeks, the preparation lost 93 per cent of its activity and after 3 months, 96 to 97 per cent. Hess and Unger (672) found no vitamine C in prunes, while orange peel was active even after 3 months. The storage of orange juice has a definite influence, since in old juice, only half of the activity remained. Dried carrots, kept for 3 to 5 weeks and for 7 months, were quite inactive. Hess and Unger concluded, from this that dried vegetables actually retain their nutritive value, though as regards vitamine C, they cannot replace the fresh natural products. The ageing applies not only to storing and drying, but also to fresh vegetables. In this respect, Hess and Unger (l.c. 631) investigated carrots of various ages. While young carrots retained their activity even after cooking and drying in a current of air, and storing for 3 months, this was not the case with old carrots.

From what has been said, we see that universal rules as to the stability of the vitamines cannot be formulated. Every single case must be investigated separately, since we do not yet know of any rule for determining the stability or lability of these substances. Evidently, the vitamine content of foodstuffs prepared for ingestion depends considerably upon its original content in the natural state. This original content may vary with the country in which it is produced, with the condition of the soil, the climate and so on. This is true to our primary sources of vitamines. As for the secondary sources, animal products, the question is much more complicated and depends, for the most part, upon known factors, but partly upon unknown factors too, which we have already discussed.

CEREALS

Under this heading we shall describe not only the cereals, such as wheat, barley, rye and maize but also the breadstuffs. Although an etiological relationship between the refining of cereals for human consumption and beriberi has been suspected for a long time, and in fact demonstrated, the economic conditions arising out of war have stimulated interest in the question and in established knowledge on the subject. In almost all countries, the investigators have endeavored to utilize the nutritive value of cereals to the best possible advantage. To the problem of the economic utilization of the grain kernels, another factor is added which we have already noted in the etiology of avian beriberi, namely, that in most cases, vitamines A and B are localized in a certain portion of the kernel. Because of this, modern refining may result in a more or less complete removal of these essential factors. It is indeed true that under normal conditions, when the population eats other foodstuffs, in addition to bread, there is no immediate danger of an avitaminosis, and yet we must remember that at present bread may constitute a large percentage of the diet. Although whole wheat bread is used almost exclusively in many countries, in more cultured sections, white bread is used in preference. Aside from the aesthetic factor, some scientific facts are known which make it desirable to mill the grain. One of the reasons frequently cited is the fact that flour from whole wheat keeps with greater difficulty. In fact, the whole grain, very rich in fat, is very difficult to store. The polishing of rice is often defended on these grounds, and Ottow (l.c. 518) proposed measures for increas-

ing its conservability. This is even more applicable to corn, so rich in fat. Winton, Burnet and Bornmann (673) state that corn may be stored much better if the germ has been removed. The second reason why it is difficult to introduce whole grain in practice, is that many investigators are of the opinion that bread baked with the chaff in uneconomic. The assimilation of this bread is supposed to be not only poor, but it occasions losses in other foodstuffs simultaneously consumed. Still, the investigators are not agreed upon this point. While Hindhede (674), Röhmann (675), Eijkman (676), Hulshoff Pol (677), Dutcher (678) and Stoklasa (679) believe that whole wheat bread is very well utilized; we also encounter views, such as those of Pugliese (680), van Leersum and Munk (681) and Rubner (682) who asserted that such breadstuffs are poorly utilized and in fact provoke losses, as has been noted above.

Since the time of Liebig (683), we have known that milling of grain decreases its nutritive value; Liebig said: "No single foodstuff loses its value so readily as whole grain through the modern process of milling. The whiter the flour the less nutritive value it possesses." In 1871, Magendie (684) knew that pigeons die when fed on fine wheat flour, while on rye, they do very well. Dogs on wheat flour die after 40 days, while on coarse "soldiers' bread" they continue to live. Oseki (685) investigated the value of various kinds of bread on mice. He found rye and army bread suitable, while wheat bread and barley flour were unsuitable. Weill and Mouriquand (686) chose to add extract of bran, instead of the bran itself, in baking bread, whereupon the addition of the occasionally objectionable cellulose was obviated. Sherman, Rouse, Allen and Woods (687) believed in the greater nutritive value of the whole wheat kernel as compared with white bread, judging from their rat experiments.

Our knowledge as to the localization of the vitamines in grain kernels has undergone some modification in recent years. While the first workers in this field stated that the protective substances are found in the "silver skin" (a thin membrane surrounding the rice kernel), Fraser and Stanton thought the protective substances were to be found in the aleurone layer. Later, McCollum and Davis (688) showed that vitamine B is found chiefly in the germ. Chick and Hume (689) found this vitamine in rice, wheat and corn, partially also in the pericarp. Voegtlin, Lake and Myers (690) believed that they could show by animal experiments that vitamine B of wheat

and maize occurs chiefly in the germ, as well as in the pericarp; in this they adopted the view of Chick and Hume at first. Later however, Voegtlin and Myers (l.c. 271) showed that when wheat or corn kernels (see fig. 53–54) are cut into two portions, and the part without the germ fed to animals, results are obtained which indicate an absolute lack of vitamine B; therefore, the vitamine must be in the germ only. However, it must not be forgotten that by the procedure followed by Voegtlin and Myers, other constituents are removed (for example, protein), and that possibly the vitamine is also present in the remaining part of the grain in sub-optimal amounts. Funk and Dubin (l.c. 493), showed that the substance promoting the growth of yeast was found in the germ, but it was likewise present outside of the germ in appreciable quantities. For practical purposes, according to Greig and Curjel (691), it seems sufficient when wheat germ is added to zwieback to the extent of 15 per cent. This measure was adopted for the English colonial army in order to protect the personnel against beriberi.

When cereal flours are heated in baking, the vitamine B content is altered. Weill and Mouriquand (692) heated decorticated barley to 120°C. for 1½ hours. When this was fed to pigeons, they died after 30 days with definite beriberi symptoms. On white rice, this disease appears in 18 to 25 days and on sterilized rice, in 9 to 16 days. They believed, therefore, that decorticated grain kernels still contained vitamine, especially since the symptoms appeared sooner when the food was sterilized. Chick and Hume (l.c. 645) heated wheat germs to high temperatures, whereupon they observed inactivation at 120°C., but not on heating at 100°C. for 2 hours. From these results, they believed that baking does not affect vitamine B to any great extent. If yeast is used in the dough, then a part of the B vitamine of the bread may arise from this source.

We have frequently touched upon the point that seeds develop vitamine C upon germination. This fact, first observed by Fürst, was further developed for practical purposes by Chick and Delf (693), and in addition to cereals, was confirmed in peas and beans. Weill and Mouriquand (694) conducted similar experiments with germinating barley and later they showed, in conjunction with Pérronet (695), that vitamine C develops in greater amounts only in the later stages of the germination.

1

2

3

FIG. 52. *1*, UNPOLISHED RICE; *2*, PARBOILED RICE; *3*, POLISHED RICE (FRASER AND STANTON)

Rice

We have already touched upon most of the data on rice, including those of Fraser and Stanton (l.c. 49), who showed by means of illustrations how the loss of substances occurs during the polishing of rice. From the appended illustration, we see why such great differences in the number of beriberi cases are apparent with the use of polished and parboiled rice. The question as to whether polished rice still contains vitamine B has likewise already been discussed. It is important now to determine whether this rice contains vitamine A, but we find no observations on this point. It would be of special interest to see whether pigeons can be maintained for a long time on polished rice with the addition of vitamine B. Funk and Dubin (696) have started experiments in which pigeons are being fed on rice to which has been added 5 per cent casein and 3 per cent salts, and the mixture autoclaved at 120°C. for 3 hours. By means of the prolonged heating, it was expected that the rest of the vitamine A would be destroyed. The pigeons have gained in weight and have remained in good health for over six months. Despite the fact that others have investigated this problem, it is not yet certain whether half grown pigeons can live without an addition of vitamine A, or whether it must be concluded that autoclaved, polished rice still contains traces of vitamine A. As regards the nutritive value of rice polishings, Mattei (697) believed that pigeons could not be maintained on rice polishings alone; he found that an addition of white rice was necessary. Wise and Broomel (698) investigated the effect of the modern milling methods on the composition of the remaining rice kernel. On polishing, the husk germ, 6 layers of bran and a part of the seventh layer were removed, so that the total loss in weight was about 10 per cent. On analysis, it was possible to show a loss of 70 per cent ash, 85 per cent fat, and 10 per cent protein. To prevent these losses, Guareschi (699) has suggested the use of rice which is not so highly milled.

Barley

According to the older statements about barley, it seems that its vitamine B is not localized in the same way as in other cereals. In the older Japanese data, for example, that of Saneyoshi (700), we find that barley can cure avian beriberi, whereas Weill and Mouriquand

(701) used decorticated barley to produce beriberi. Steenbock, Kent and Gross (702) observed that for rats, barley, like other cereals, was deficient in vitamine A, protein and salts.

Oats

McCollum, Simmonds and Pitz (l.c. 372) regarded the composition of oats as unfavorable for the nutrition of animals. Tested on rats, it showed a lack of vitamine A, protein and salts. This applies much less to pigeons, as we ourselves have shown.

Wheat

Voegtlin and Myers (l.c. 271) believed that vitamine is found exclusively in the germ; we have already published our findings in this respect (l.c. 271). How the various parts of the wheat kernel are related to each other, we may see from the following diagram, taken from the work of the above investigators, from which we may note the method of sectioning used by them in their experiment on the localization of vitamine B. McCollum, Simmonds and Pitz (703) investigated, in particular, the vitamine value of wheat germ. Qualitatively considered, this portion of the wheat has been shown to be adequate, but for practical reasons, an addition of salts must be given. Vitamine B occurs in the germ in large quantities; vitamine A, on the other hand, in smaller amounts. Osborne and Mendel (704) investigated the nutritive value of various milling products of wheat kernels, and did not believe in the exclusive localization of vitamine B in the germ. The contiguous portion was also rich in vitamine B, as was also the periphery of the kernel, the endosperm and bran not containing very much.

FIG. 53. WHEAT KERNEL SHOWING DIFFERENT LAYERS (VOEGTLIN-MYERS)

Maize

Because of a possible etiological relationship of corn consumption to pellagra, the numerous investigations on the nutritive value and composition of corn and corn products are of special importance. Bezzola (705) showed that corn does not provide an adequate diet for the maintenance of guinea pigs in good health; they lose hair, develop severe diarrhea and subsequently die. A difference between the use of good and bad corn was not observed. Lucksch (706) found that guinea pigs, fed on good corn, lost their hair, showed a hyperemia of the intestinal mucosa and an enlargement of the adrenals. On a mixture of corn, flour and greens, they lost hair nevertheless, and developed paralysis of the hind legs and catarrh of the small intestine. Corn was likewise shown to be inadequate for rabbits and dogs, the results obtained in spring being more definite than those in autumn. The blood of the experimental animals was investigated and found sterile at all times. v. Neusser (707) reported on a disease of horses, called "emmaisadura" in Mexico and Colombia supposed to be caused by rotted corn. The symptoms of this disease are emaciation, dizziness, lethargy, cramps, wildness, loss of hair and teeth and decay of the hoofs. Holst (708) remarked on the frequent occurrence of scorbutic symptoms in pellagra, especially the porosity of the bones. He repeated Lucksch's experiments with guinea pigs and came to the conclusion that the disease described by him could be prevented by the addition of fresh cabbage. In particular, the constant loss of hair could be overcome although the experiments were carried out in spring. The whole syndrowe was thereupon regarded by Holst as scurvy. Baglioni (709) likewise carried out some feeding experiments with corn products on guinea pigs. In the light of modern investigations, Holst was right when he characterized as scurvy, the disease developed by guinea pigs fed on corn. Since in most corn-feeding experiments, no antiscorbutic was given, the resulting condition was complicated by scorbutic symptoms to such an extent, that these investigations are of no value in the explanation of pellagra. The problem appears in a different light when birds are used as experimental animals. Ohler (l.c. 262) observed that when decorticated maize is fed to chickens, they

develop beriberi. Under the same experimental conditions, Driscoll (710) noted a condition which he thought was similar to pellagra in man; among other symptoms, he observed an erythema of the legs; the chickens recovered when given whole corn. Clementi (711) fed chickens on cooked corn and "polenta" and observed a development of nervous and digestive disturbances, but only after many months of feeding. Szalagyi and Kriwuscha (712) also conducted some corn feeding experiments with chickens, ducks and geese.

Urbeanu (713) carried out some rat and chicken experiments, and noted some skin symptoms in both. In the former, this was apparent only after 10 to 12 months of feeding, and was favorably influenced by the addition of potatoes and soya beans. Suárez (714), experimenting with pigeons and mice, observed in both a disease similar to beriberi after 25 to 35 days, which was favorably affected by yeast. Experimenting with dogs, Nitzesco (715) stated that corn was not a complete food. Rondoni (716) attempted to use guinea pigs in his pellagra work, but recognized that these animals were unsuited for this purpose, and therefore suggested that the work be continued on man and monkeys.

Hogan (717) fed corn simultaneously to rats and pigs; in the former, this diet was very nearly sufficient, but in the latter, a lack of protein was noted, since on adding egg-albumin, growth was resumed. This brings us to a consideration of the nutritive value of corn proteins, a subject which has assumed considerable significance in the etiology of pellagra. In this connection, Mendel and Fine (718) carried out a series of dog experiments. According to their results, corn glutellin, a preparation consisting of zein and glutenin, seemed to be as well utilized as meat. The relationship between the two protein components varied within certain limits, but averaged 1:1. The proteins were separated by means of alcohol, in which zein is soluble. Osborne and Mendel (719) and Baglioni (720) conducted experiments on the nutritive value of corn protein. The utilization was better with the flour than with the proteins isolated. All three products however gave a nitrogen retention, and it would therefore be concluded that corn also contains proteins of a higher nutritive value than that of zein. McCollum and Simmonds (721) found that when rats are fed on a mixture of 80 per cent corn and 20 per cent beans, there is still evidence of a lack of vitamine A and protein.

The investigations of Johns, Finks and Paul (722) gave the following percentage composition of corn as regards various protein compounds:

Globulin	21.9
Zein	41.4
Glutellin	30.8
Alcohol-insoluble part	5.9

While it is known that zein is not a complete protein, the composition of glutellin shows it to be of good nutritive value. As an explanation of the poor nutrition resulting from an exclusive corn diet, these investigators stated that certain flour products lack, first of all, vitamine B; second, although they contain complete proteins, they occur in dilute form, so that the protein must be added as concentrates. It is interesting to note that this view corresponds exactly with our present conception of the matter. Osborne and Mendel (723) found that the dietary deficiency arising from corn meal feeding is caused by a deficiency of protein. This deficiency may be corrected by an addition of skimmed milk powder, which must amount to 50 per cent of the corn fed, before the nutritive condition is restored to normal.

We come now to a consideration of the vitamine content of corn, and how it is affected by milling. It has been known for a long time that corn germ contains much more fat than the whole corn. The appended table is taken from the work of Woods (724). The protein content varies in the same way.

	FAT	PROTEIN
	per cent	*per cent*
Whole corn	4.3	12.7
Endosperm	1.5	12.2
Husk	1.6	6.6
Germ	28.6	21.7

We have personally made analyses of various South Africa corn products (l.c. 525) and concluded that milling causes considerable losses, which may be prevented. Our figures on milling are recorded below.

Data pertaining to milled maize (97 per cent of the total grain)

	WATER	ASH	NITROGEN				PHOSPHORUS: P_2O_5	FAT	FATTY ACIDS	CHOLESTEROL	LIPOID-P_2O_5	COLOR REACTION. FOLIN AND MACALLUM	
			Kjeldahl	Dumas	Melanin	Van Slyke						Alcoholic extract	
												Hot	Cold
1. Whole maize grain	12.71	1.56	1.73	1.74	0.14	0.99	0.56	4.5	3.88	0.247	0.0154	0.35	0.55
2. Highly milled meal 86 per cent	12.63	1.48	1.67	1.73	0.13	0.95	0.36	3.87	3.36	0.22	0.0098	0.23	0.45
3. First milling from no. 2	10.48	2.09	1.23		0.14	0.61	0.30	4.66	4.18	0.357	0.0164	1.00	1.00
4. Second milling from no. 2	10.71	4.04	2.31	2.34	0.16	1.40	1.43	12.79	11.09	0.438	0.0353	0.70	0.60
5. Slightly milled meal, 97 per cent	12.41	1.60	1.73	1.84	0.12	1.00	0.54	4.21	3.63	0.233	0.0153	0.30	0.45
6. Bran from 5, above	10.55	1.40	0.65		0.07	0.30	0.23	1.86	1.58	0.271	0.0084	0.35	0.70

We have found nothing in the literature pertaining to the nutritive value of the proteins of different corn layers. It is, of course, possible that the two chief proteins of corn—zein and glutenin—are localized in various layers, so that milling may deprive the corn of glutenin, and hence enrich it with the less valuable zein. With the above analyzed products, we have carried out feeding experiments on pigeons, but have never observed beriberi. From the diagram of the corn kernel (fig. 54), it is possible to see the localization of the germ, explaining the loss of substance and nutritive value during milling. Analyses of corn milling products were also published by Juritz (725) and by McCrae (726). Poppe (727) found, besides this, that by steeping the corn, similar to cooking, the nutritive substances extracted by the water amount to 36.2 per cent. McCollum, Simmonds and Pitz (728) found that corn contains all of the necessary

amino acids, but not in the proper proportion to each other and, in addition, was poor in vitamine A and salts. While this was found to be the case in rats, Weill and Mouriquand (729) were able to maintain pigeons on whole corn for more than 240 days, whereas decorticated or sterilized corn produced severe disturbances. Voegtlin, Lake and Myers (1.c. 690) found that whole corn contains sufficient vitamine B for pigeons. However, if they fed the same corn with the biggest part of the aleurone layer and germ removed the pigeons and chickens developed, in about 3 weeks, a disease similar to beriberi which could be cured by giving vitamine B. Voegtlin, Sullivan and Myers (730), as well as Hughes (731), also obtained similar results.

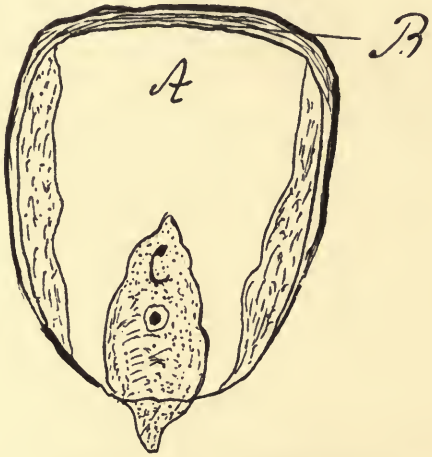


FIG. 54. CROSS-SECTION OF CORN KERNEL

A, endosperm., *B*, husk, *C*, germ

Steenbock and Boutwell (l.c. 588) showed, contrary to the ruling view that corn is poor in vitamine A, that certain strongly yellow colored varieties are quite rich in this vitamine. In connection with this, we wish to emphasize once more that corn is a complete diet for most species of birds. It is different with mammals, for which corn is not so favorable, and this fact is now generally attributed to the low biological value of the corn proteins, although, inasmuch as corn contains a protein that is adequate for birds, it is incorrect to speak of the low biological value of corn proteins. In experimenting with mammals, particularly those that can develop scurvy, we must not forget that corn, at least when dry, contains no C vitamine. This

minimal value of corn proteins does not depend so much on the unfavorable content of amino acids, as that for some animals, the dilution of the complete proteins by the incomplete, and by large amounts of starch, has an unfavorable effect on the utilization.

POTATOES

We have already stated that in a population that is nourished chiefly on potatoes, no avitaminoses appear and pellagra is unknown. The value of potatoes as a protection against deficiency diseases has, to a certain extent, been recognized by some investigators. Although the protein content of potatoes is small, and although large quantities must be used because of the great dilution of the more valuable components present, it seems to be certain that this dilution is harmless to people. The author has often had the opportunity of observing Polish peasants at their meals. When the men returned from the fields, an enormous plate of potatoes was set before them, so that a city dweller could hardly conceive of the possibility of consuming such a great amount of food. Still, the entire contents of the dish (not plate), cooked with a trace of lard to give added taste, disappeared in an unbelievably short time. From the many modern investigations on the nutritive value of potatoes, it is clear to us why these peasants must consume such an unusual amount of potatoes. It is correlated with the small content of protein, together with the three known vitamines. The splendid utilization of potato proteins was unquestionably demonstrated by Hindhede (732), and we shall discuss this later on. His findings were confirmed by Abderhalden, Fodor and Röse (733) as well as by Rose and Cooper (734). Despite the work of Rubner (735), we believe that Hindhede's results are on a firm foundation.

The experiments with animals, especially rats and guinea pigs, resulted differently from those on man. We have already seen in the author's earlier work on guinea pig scurvy, that fresh potato juice did not possess any great protective action against this disease. McCollum, Simmonds and Parsons (736), experimenting with rats, obtained results quite dissimilar to those in man. They found that potatoes cannot take the place of other grains in their nutritive value, and are deficient in salts, protein and vitamine A. One would be tempted to conclude from this that, between rats and man, there are differences in the utilization of potatoes. Osborne and Mendel (737)

found that the vitamine B content of potatoes is sufficient for rats, and no difference was apparent between new and old potatoes. Abderhalden and Schaumann (l.c. 499) made the observation that when pigeons are fed on dried potatoes, they develop edema on the lower part of the legs. As regards the vitamine content, Chick and her co-workers (l.c. 333) found that potatoes (cooked and raw) contain all three vitamines in small quantities. Boruttau (738) studied the effect of temperature on the nutritive value of potatoes in mice. Similar experiments were made by Auer (739) in Hofmeister's laboratory. The effect of drying was studied by Givens and McClugage (l.c. 657).

MILK

Milk is such an important food for life that we must describe it more in detail. With regard to the milk question, we were first to suggest the relationship between the composition of the milk and some deficiency diseases in children. In 1912, we stated two factors, without which the explanation of certain avitaminoses would be impossible. The two essentials are first, that on heating the milk, certain vitamines become more or less completely destroyed, and second, that the vitamine content of milk must depend on the vitamine content of the fodder. Although both are self-evident, they gained recognition only with difficulty. The following rules, laid down by us at that time for feeding of milk to children, are still of value at present: *Hygiene of the cow, good fodder, least possible boiling of the milk, least possible storage after milking and after pasteurization.* Our uncompromising attitude on the milk question was made necessary by the fact that Lane-Claypon (740), in a report to the Local Government Board in London, came to the conclusion that boiling does not affect the nutritive value of milk. As a matter of fact, the same investigator (741), one year later—1913—declared that the question needed further development. The ensuing years have brought to light a great number of investigations which corroborated our view on the subject completely. Later, Lane-Claypon (742) published a book dealing with the question in detail.

Demonstration of vitamines in milk

In 1912 we (l.c. 324), proved the presence of vitamine B in milk, and Hopkins also discovered the presence of a growth substance.

The description of infantile beriberi by Hirota (743) and Andrews (744) showed, of course, that the vitamines in milk must originate in the food of the mother, since these young ones were nursed by mothers suffering from beriberi. The question as to the nutritive value of heated milk, especially in relation to infantile scurvy and without knowledge of vitamines, was treated by Sir Thomas Barlow, Neumann, Heubner and others; we shall discuss this work in detail in the chapter on scurvy.

Influence of food on the vitamine content and composition of milk

Because of the proof of the variation in the composition of milk, we are in position to explain some of the divergent statements in the literature. These facts, long known to cattle breeders and milk producers, of course without any knowledge of vitamines, must be proven anew from the viewpoint of the vitamines. That the milk may possess a different nutritive value according to the fodder, has been shown by Carr, Spitzer, Caldwell and Anderson (745), Hart, Nelson and Pitz (746), McCollum and Simmonds (747) and Drummond (748). A series of interesting experiments were published by Eckles, Palmer and Swett (749–750). The latter showed that the composition of milk is not constant; especially does the protein and fat content vary considerably. The recognition of the variation in the protein content of milk bears some relationship to our modern conception of pellagra—which we shall discuss further under the subject of pellagra—since under these conditions infantile pellagra should be more frequently encountered, which is not the case. The variation in the composition of milk explains the results of Chick, Hume and Skelton (751), who found that for the protection of guinea pigs against scurvy, a larger quantity of milk than that found by us was necessary. The question as to the origin of milk vitamines from the food has already been treated experimentally. McCollum, Simmonds and Pitz (752) accepted this view for the vitamine A and B content of milk. Osborne and Mendel (l.c. 326) observed no difference in the vitamine B content of summer and winter milk. Regarding vitamine C, this relationship is very apparent. Hart, Steenbock and Ellis (753) fed cows on dry and fresh fodder. While of the milk resulting from the fresh fodder, 15 to 50 cc. daily were necessary to protect guinea pigs from scurvy, of that arising from the dry fodder, 50 to 75 cc. were necessary to produce the same result.

These findings were confirmed by Hess, Unger and Supplee (754), who showed further that even a 3 weeks feeding with dry and fresh food was sufficient to show the above variations. Guinea pigs lived an average of 56 days on milk produced on the dry fodder, while on the milk resulting from the fresh fodder the animals lived more than 120 days, and after this time showed only a mild scurvy. In the milk containing more vitamine C, more calcium and phosphorus and 50 per cent more citric acid were found. Dutcher and co-workers (754a) corroborated the above findings as to the dependence of the vitamine content of the milk on the vitamine content of the cow's food. According to Steenbock, Boutwell and Kent (l.c. 594) the same is likewise true of vitamine A, after only a 3 weeks' feeding.

The vitamine content of milk

Inasmuch as we have seen that the vitamine content of milk varies according to the composition of the diet,[5] we must add that the vitamine content even of raw milk is not very high. Therefore it is obvious that any treatment of milk, or merely storing, may easily diminish the margin of safety. The author (l.c. 325) as well as Osborne and Mendel (l.c. 326) and Gibson and Conception (755) showed that the vitamine content of milk is not very high. That this is also true of vitamine C was demonstrated by Chick, Hume and Skelton (756). As regards vitamine A, it seems to occur in larger quantities; still, we are rather uncertain of its stability, since every manipulation, because of the apparently easy oxidizability of the substance, may have an effect on the nutritive value of the milk.

We may readily conceive, from the above, that the treatment of milk may produce a deficiency disease in children whose practically sole food it is. That this is the case in infantile scurvy, was shown by Hess (757) using pasteurized milk. The disease developed after a few months and was only of a mild nature. Hess (758) and Hess and Unger (759) correctly observed that, aside from heating, storage plays a great part in the conservation of antiscorbutic power. Miller (760) was also of the opinion that since raw milk contains only a small quantity of vitamine C, just within the margin of safety, further manipulation may be dangerous.

[5] In addition, Moore (754b) raised the question as to whether infectious conditions of the cows may not influence the vitamine content of milk.

The influence of heat on the vitamine content of milk

Practically all of the studies related to this point were made with vitamine C. In this respect, most workers agree that the use of heated milk may result in scurvy. Some investigators, on the contrary, believe that the decrease in the nutritive value after heating cannot be attributed entirely to the vitamines. McCollum and Davis (l.c. 97) stated that this is related to an injury of the casein by heat. We have already mentioned this explanation and experimentally disproven it. Daniels and Stuessy (761) showed that rats fed on milk, cooked for 1 minute, could not grow. This experiment was repeated, varying the temperature and time of heating. They found, at the time, that an addition of protein, in the form of casein or egg-yolk, permitted the rats to resume growth. In a recent paper, Daniels and Loughlin (762) report that the results of Daniels-Stuessy with casein and egg-yolk were to be attributed simply to an addition of calcium salts. They found that when milk is heated slowly at a lower temperature, as in pasteurization, a deposit containing calcium salts is formed in the kettle. If the milk is quickly boiled up, or if care is taken to mix the above deposit in with the milk, then the rats grow just as well as on unheated milk. The same results were obtained when soluble calcium salts, like calcium glycerophosphate, were added to the heated milk. Regarding the significance of the results of Daniels and Loughlin critically, they maintain that the decrease in the nutritive value of milk after heating is due, not to the destruction of B- and A-vitamines, but to the precipitation of calcium salts. Although the destruction of vitamine C is not touched upon in this work, the investigators wished to convey the impression that the changes of the milk on cooking had nothing to do with the vitamines. It is not impossible, however, that if they would repeat their work, using children, as they proposed to do, the results would be different from those with rats.

The first investigation on the influence of heating on vitamine C was made by Frölich (763). Milk heated at 98° C. for 10 minutes lost its protective action against guinea pig scurvy, while heating at 70° C. for 30 minutes gave uncertain results. Whereas the author found that 50 cc. milk prevented scurvy in guinea pigs, Chick, Hume and Skelton (l.c. 751) observed that this was not sufficient. Barnes and Hume (764) investigated the effect of heating, drying and rapid

boiling on the vitamine content of milk. Dried milk (dried rapidly at a high temperature) decreased markedly in its vitamine C content and was inferior to milk boiled for a short time. The activity of vitamines A and B seemed to be unaffected by drying. Hart, Steenbock and Smith (765) obtained similar results with raw and evaporated milk.

Influence of drying and evaporation on milk

From an early "Report to the Local Government Board" (766), we see that vigorous objections were raised against the nutritive value of dried milk. In particular, the dilution necessary before consumption was regarded as fraught with danger. This important question was officially investigated in the United States and in England, and a report on this subject appeared in the United States Public Health Reports (767). Winfield (768) studied this question, using 87 children and 40 rats, and came to the conclusion that dried milk seems to be complete, but only for the first growth period. The rats did not grow any more after they reached two-thirds of their size, and the usual mixed diet had to be resorted to at this point. Naish (769) stated that in the use of dried skimmed milk for feeding children the danger of scurvy does not exist, even without fruit addition, while Pritchard (770) recommended the addition of fruit. Hess and Unger (l.c. 631) are of the opinion that milk, dried according to the Just-Hatmaker process for a few seconds at 116°C., is protective against scurvy equally as well as fresh milk. In a few cases, a cure could even be brought about. Sekine (771) conducted some experiments with condensed milk and showed that young mice remained normal on this diet for about 100 days, after which they suffered from beriberi and anemia. On the addition of iron and vitamine B, they again became normal. Coutts (772) found that dried milk gave better results in the nutrition of children than did fresh milk. He believed that 3 months old infants could tolerate dried whole milk. He recommended also a synthetic milk, in which the cream was substituted by cheaper vegetable fats which were, however, apparently poor in vitamine A. Gerstenberger (773) and his co-workers tried to replace the butter-fat by a cheaper fat. They started with skimmed milk to which were added vegetable fat and cod liver oil. This milk was fed to 311 children, who subsequently showed no signs of rickets, spasmophilia or anemia.

General remarks on the nutritive value of milk

The question of dilution in connection with the use of condensed and dried milk appears also to be of some practical significance. Washburn and Jones (774) have shown that the fat content of milk is likewise of great significance. Working with young pigs, they demonstrated that skimmed milk, as such, does not permit of growth in these animals. Milk with a moderate amount of fat (2.5 per cent) seemed to give the best results, while too much fat (5 per cent) produced rapid growth, the animals being too fat and apathetic. Too great a dilution of the milk was followed by a considerable decrease in its nutritive value, while a very concentrated milk reared young that were excessively fat and weak, the bones showing only two-thirds of their normal strength. Lately, views have appeared which emphasize the exceptional position of milk in nutrition. Freise (l.c. 342), as a result of some rat experiments, expressed the view that the milk was either lacking an important constituent or had lost it during manipulation. He observed, however, that animals on a deficient diet recovered rapidly when an addition of 10 per cent dried milk was given, so that milk possesses a splendid supplementary action. The same characteristic is manifested by barley-malt. Honey, cereals, alcoholic extracts of barley-malt, carrots, egg-yolk, beans, casein and smaller amounts of dried milk (less than 10 per cent) no longer show the activity of dried milk. Mattill and Conklin (775), working with rats, obtained the same results. On ordinary milk the animals grew well at first, but later, especially in 50 to 100 days old females, growth ceased. Autolyzed and ordinary yeast and wheat germ were added, resulting in only temporary improvement; addition of iron and casein was without effect. On the other hand, better results were obtained on adding dried milk, showing that the dilution of the foodstuff given is of significance. Dried milk was shown not to be the ideal food for later stages of life. Here again, a dilution (55 parts dried milk, 40 parts starch and 5 parts butter) was of help resulting in normal growth and reproduction. The dilution of dried milk with lard gave the same results as with starch. Addition of 1 per cent yeast resulted in normal growth. For these reasons, the authors raised the question, whether the addition of yeast does not introduce something that is missing in the other combinations, and proposed new

experiments to clear up this point. From what has been said in this chapter, it is evident that we are not yet fully informed as to the nutritive value of milk, and that this foodstuff still presents some riddles for us to solve.

MEAT

The significance of meat as a component of our diet is threefold. First, as an important source of protein; second, as a source of vitamines when it is used as a sole food; and third, for its "vitamine-sparing" action. The last characteristic will be treated more fully later. In accord with our own experience, we know of no single food which requires so little vitamine addition as does protein material, especially meat.

When we speak of meat in this chapter, we have in mind muscle tissue only, since glandular organs like liver, kidney, etc., are characterized by a special richness in vitamines. Vitamine B was first demonstrated in meat by Cooper (l.c. 535). Osborne and Mendel (776) investigated the value of dried meat and did not find it particularly rich in vitamine; on successive extraction with water, the amount of vitamine B decreased considerably. According to Osborne and Mendel (777), dried meat contains sufficient vitamine A, while pig's brain and heart has enough of both A- and B-vitamines. Cole (778) reported that powdered meat contains a sufficient amount of A- and B-vitamines.

Pertaining to the vitamine C content of meat, practical experience and laboratory findings do not seem to go parallel. Naturally, the discrepancies are only apparent, since practice has shown that fresh meat contains small amounts of vitamine C. This is evident from the report of Nansen (779) on his polar expedition, as well as from the report of Stefánsson (780). The latter stated that scurvy was cured by an addition of fresh meat. However, we already knew from the work of Curran (781) that 2 to 4 pounds of raw meat are necessary, since he observed cases of scurvy on a daily consumption of 500 grams cooked meat. Willcox (782) stated that in the English army in Mesopotamia scurvy developed because the Indian troops refused to eat meat. He was of the opinion that meat could function as an antiscorbutic. Pitz (l.c. 365), in his studies on guinea pig scurvy, found that an increase in the protein proportion of the diet definitely prolongs the life of the animal, which may perhaps be regarded as

the "vitamine-sparing action" of protein. Dutcher, Pierson and Biester (783) found that guinea pigs could not be protected against scurvy by an addition of fresh meat juice. This was also shown by Givens and McClugage (784) with dehydrated meat; however, these results may be criticized on the basis that guinea pigs need more vitamine C than does man. That the vitamine requirements of different animals may vary has already been demonstrated by Harden and Zilva (l.c. 455).

Parsons (l.c. 191a) showed, on the contrary, that some organs, especially the liver, but also spleen and kidney, contain relatively large amounts of vitamine C—so much so, that 10 grams of pig's liver per day is sufficient to protect guinea pigs from scurvy. By the preparation of extracts, instead of juices or organs themselves, Parsons was able to add larger quantities of extractives from the muscle, showing that an extract of 55 to 95 grams of muscle suffices to protect guinea pigs from scurvy. Fish muscle possessed no anti-scorbutic property.

THE VITAMINE CONTENT OF THE MOST FREQUENTLY USED FOODSTUFFS

The compilation, which, as we are well aware, cannot be complete, gives the reader an idea of the number of investigations carried out for the demonstration of the present known facts. We have not sought to give the figures for the vitamine content since we believe that sufficient progress has not yet been made. In the table, a single plus sign denotes that the respective vitamine is present only in traces. With regard to protein, the plus sign indicates that it is not adequate for nutrition. From the table, we may see that those starting materials which are very rich in vitamines contain proteins of high biological value.

PRODUCTS	B-VITAMINE	LITERATURE	A-VITAMINE	LITERATURE	C-VITAMINE	LITERATURE	PROTEIN	LITERATURE
Cereals, flours and grasses								
Oats	++	McCollum-Simmonds-Pitz (372)	+	McCollum-Simmonds Pitz (372)			+	McCollum-Simmonds-Pitz (372)
Oatmeal	+	Oseki (685)	0	McCollum-Davis (785)				
Rye			0	McCollum-Davis (785)			++	Abderhalden (789)
Ryebread	++	Oseki (685)					++	Oseki (685)
Barley	++	Steenbock-Kent-Gross (702)	0	Steenbock-Kent-Gross (702)			+	Steenbock-Kent-Gross (702)
		Saneyoshi (700)						
Barley flour	0	Oseki (685)						
Barley, germinated					++	Weill-Mouriquand (694)		
Wheat, whole							++	Hindhede (791)
Wheat, bran	++	Sullivan-Voegtlin (506)						
Wheat, germinated	+++	Chick-Hume (689)	0	McCollum-Simmonds-Pitz (703)	0	Hess (792)	++	McCollum-Simmonds-Pitz (703)
	+++	McCollum-Simmonds-Pitz (703)						
		Osborne-Mendel (704)						
Wheat bread	0	Oseki (685)					++	Sherman (793)
Wheat flour							++	Baglioni (720)
Wheat endosperm	+	Funk-Dubin (493)						
	+	Osborne-Mendel (704)						
	+	Chick-Hume (689)						
Army bread	++	Oseki (685)						
Rice, whole	++						++	Suzuki (323)
Rice, polished	0		0?				+	Suzuki (323)
Rice, polished, autoclaved	0							
Rice, steamed	++							
Corn, whole	+++	McCollum-Simmonds-Pitz (728)	+	McCollum-Simmonds-Pitz (728)				
Corn, germinated	++++	Chick-Hume (689)						
Corn, endosperm	+	Chick-Hume (689)						

Corn, fodder			++	McCollum-Davis (785)				
Corn, gluten	+	Johns-Finks-Paul 722)						
Corn, yellow	+++	Johns-Finks-Paul (722)	++	Steenbock-Boutwell (588)				
Corn, yellow, autoclaved			++	Steenbock-Boutwell (598)				
Corn, white			0	Steenbock-Boutwell (588)				
Corn, meal	+	Oseki (685)					+	Baglioni (720)
Corn, oil			++	Drummond-Coward (592)				
Mellin's Food	+?	Wheeler-Biester (786)					0	Wheeler-Biester (786–787)
Eskay's Food	+?	Wheeler-Biester (787)					0	Wheeler-Biester, (786–787)
Buckwheat	+	Auer (739)						
Buckwheat, husked	0	Auer (739)						
Clover, dried	++	Osborne-Mendel (788)	+++	Osborne-Mendel (788)				
Clover, ether extract			++	Osborne-Mendel (584)				
Alfalfa, dried	++	Osborne-Mendel (788)	+++	Osborne-Mendel (788)				
Alfalfa, alcohol extract			++	Steenbock-Boutwell (586)				
Alfalfa, benzol extract			++	Steenbock-Boutwell (586)				
Alfalfa, autoclaved			+	Steenbock-Boutwell (598)				
Timothy	++	Osborne-Mendel (794)	+++	Osborne-Mendel (788)				
				Legumes				
Soya bean	+++	Osborne-Mendel (794)	?	Osborne-Mendel (794)			++	Osborne-Mendel (794)
	+++	Daniels-Nichols (795)	+	Daniels-Nichols (795)			+	Abderhalden (789)
							++	Holmes (803)
							++	Daniels-Nichols (795)
Soya bean flour					0	Cohen-Mendel (374)		
Soya bean fodder			+	Osborne-Mendel (796)				
Peanuts	++	Mackenzie Wallis (797)					++	Mackenzie Wallis (797)
	++	Daniels-Loughlin (798)	+	Daniels-Loughlin (798)			++	Daniels-Loughlin (798)
	++	Greig (799)	+?	Coward-Drummond (605)			++	Johns (804)
Peanuts, steamed							++	Holmes (803)
Peanuts, oil			+	Drummond-Coward (605)				
Peas	+++	Sullivan-Voegtlin (506)					+	McCollum-Simmonds-Parsons (805)
Peas, flour	+	Oseki (685)						
Peas, purée	++	Hulshoff Pol (670)						
Peas, old	0	Hulshoff Pol (670)						

PRODUCTS	B-VITAMINE	LITERATURE	A-VITAMINE	LITERATURE	C-VITAMINE	LITERATURE	PROTEIN	LITERATURE
Peas, germinated					++	Chick-Delf (693)		
Beans, old	0	Hulshoff Pol (670)						
Beans, germinated					−−	Wiltshire (806)		
Beans, flour	+	Oseki (685)						
Beans, autoclaved	++	Daniels-McClurg (654)						
Horse beans							+	Abderhalden (789)
Lupine beans							+	Abderhalden (789)
Lentels	++	Cooper (643)						
Lentels, germinated					++	Chick-Delf (693)		
Runner beans, pods					+++	Campbell-Chick (669)		
Runner beans, autoclaved					+	Campbell-Chick (669)		
Millet			Trace	Auer (739)				
White beans	+	McCollum-Simmonds-Pitz (800)	+	McCollum-Simmonds-Pitz (800)			+	McCollum-Simmonds-Pitz (800)
Seeds								
Cottonseed meal	++	Richardson-Green (801)	++	Richardson-Green (801)			++	Richardson-Green (801)
Cottonseed fodder	+?	Richardson-Green (802)	+?	Richardson-Green (802)			0	Richardson-Green (802)
							0	Wells-Ewing (807)
							++	Osborne-Mendel (808)
Cottonseed oil			+	Drummond-Coward (592)				
			+	Daniels-Loughlin (87)				
Cottonseed oil, reduced			0	Drummond-Coward (592)				
Sesame oil			0	Schabad-Sorochowetz (38)				
			+	Drummond-Coward (592)				
Linseed oil			+	Drummond-Coward (592)				
Linseed oil, reduced			0	Drummond-Coward (592)				
Vegetables								
Cabbage, raw	+	Steenbock-Gross (809)	+	Steenbock-Gross (809)	+++	Givens-Cohen (663)		
Cabbage, dried slowly	++	Shorten-Ray (665)			0	Givens-Cohen (663)		

Cabbage, dried rapidly...	++	Osborne-Mendel (788)	++	Osborne-Mendel (788)	+	Givens-Cohen (663)		
					+	Shorten-Ray (665)		
					+	Cohen-Mendel (374)		
Cabbage, cooked.....	++	Daniels-McClurg (654)			+	Delf (651)		
Cabbage juice heated....					0	Delf (651)		
Cabbage, dried stored....					0?	Delf-Skelton (671)		
Cabbage, specially dried .					++	Holst-Frölich (663a)		
Cabbage, canned.....	++	Daniels-McClurg (654)			+	Campbell-Chick (669)		
Potato, raw.....					+++	Givens-McClugage (657)		
Potato, cooked.....	++	Steenbock-Gross (810)	++?	Steenbock-Gross (810)	++	Givens-McClugage (657)	+	McCollum-Simmonds-Parsons (736)
	++	Osborne-Mendel (737)	+	Osborne-Mendel (600)				
			+?	McCollum-Simmonds-Parsons (736)			+++	Hindhede (732)
Potato juice, raw.....					+++	Chick-Hume-Skelton (756)		
Potato flakes.....	0	Auer (739)						
Potato, dried.....					+	Givens-McClugage (657)		
Potato peels.....	++	Osborne-Mendel (737)			0	Givens-McClugage (657)		
Potato, baked.....					++	Givens-McClugage (657)		
Potato, steamed.....					+	Givens-McClugage (657)		
Potato, dried slowly.....					0	Givens-McClugage (657)		
Potato, dried rapidly.....					+	Givens-McClugage (657)		
Turnip, white.....	++	Osborne-Mendel (811)						
Turnip, juice (Swede)....					+++	Chick-Rhodes (812)		
Turnip, cooked.....					++	Delf (651)		
Beet root.....	++	Osborne-Mendel (811)			0	Chick-Rhodes (812)		
Sugar beet.....	0	Steenbock-Gross (809)	+?	Steenbock-Gross (809)				
Carrots, new dried.....	+	Steenbock-Gross (809)	+	Steenbock-Gross (809)	+++	Hess-Unger (631)		
Carrots, old dried.....					++	Hess-Unger (631)		
Carrots, sun dried.....	++	Shorten-Ray (665)			++	Shorten-Ray (665)		
Carrots, dried, stored					++	Hess-Unger (631)		
Carrots, juice.....					++	Chick-Rhodes (812)		
Carrots, alcohol extract...	++	Sugiura (501)						
Carrots, ether extract.....			++	Zilva (585)				
Tomato, raw.....	++	Osborne-Mendel (811)	++	Osborne-Mendel (600)	+++	Givens-McClugage (652) (664)		

PRODUCTS	B-VITAMINE	LITERATURE	A-VITAMINE	LITERATURE	C-VITAMINE	LITERATURE	PROTEIN	LITERATURE
Tomato, heated					++	Givens-McClugage (652) (664)		
Tomato, dried					++	Givens-McClugage (652) (664)		
Tomato, canned					+	Givens-McClugage (652) (664)		
					++	Hess-Unger (668)		
Onion, raw	++	Osborne-Mendel (811)						
Onions, sun dried	++	Shorten-Ray (665)			++	Shorten-Ray (665)		
Onion, extract	0	Chamberlain-Vedder-Williams (64)						
Rutabaga	++	Steenbock-Gross (809)	+?	Steenbock-Gross (810)				
Dasheen	++	Steenbock-Gross (810)	+?	Steenbock-Gross (810)				
Mangelwurtzel	+	Steenbock-Gross (810)	+?	Steenbock-Gross (810)	+	McLean (817)		
Parsnip	++	Steenbock-Gross (810)	+?	Steenbock-Gross (810)				
Spinach, dried	++	Osborne-Mendel (788)	+++	Osborne-Mendel (788)				
Spinach, sun dried	0	Shorten-Ray (665)			0	Shorten-Ray (665)		
Spinach, ether extract			++	Osborne-Mendel (584)				
Sweet potato	+	Steenbock-Gross (810)	++	Steenbock-Gross (810)				
Lettuce	+?	Steenbock-Gross (809)	+	Steenbock-Gross (809)				
Chard	+	Steenbock-Gross (809)	+	Steenbock-Gross (809)				
Chard, autoclaved			+	Steenbock-Boutwell (598)				
Rhubarb					++	Pierson and Dutcher (1121)		
				Fruits				
Orange juice	++	Osborne-Mendel (92)	?	Osborne-Mendel (92)	++++	Delf (651)		
Orange, cooked					+++	Delf (651)		
Orange, autoclaved					++	Delf (651)		
Orange, dried	++	Osborne-Mendel (92)						
Orange, peels	++	Osborne-Mendel (92)			++	Hess-Unger (637)		

Orange, old					+	Hess-Unger (672)		
Limes, fresh					+	Chick-Hume-Skelton (818)		
Limes, canned					0	Chick-Hume-Skelton (818)		
Lemons	++	Osborne-Mendel (92)			++++	Chick-Hume-Skelton (818)		
Grapefruit	++	Osborne-Mendel (92)						
Grape juice, commercial	?	Osborne-Mendel (92)			+	Chick-Rhodes (812)		
Prunes	++	Osborne-Mendel (92)			+	Hess (819)		
Apples	+	Osborne-Mendel (92)						
Pears	+	Osborne-Mendel (92)						
Bananas	+	Sugiura-Benedict (108)	+	Sugiura-Benedict (108)	+	Lewis (820)	0	Sugiura-Benedict (108)
Tamarind					+	Chick-Hume-Skelton (821)		
Cocum					+	Chick-Hume-Skelton (821)		
Mango (amchur)					+	Chick-Hume-Skelton (821)		

Nuts

Cocoanut, presscake	++	Johns-Finks-Paul (813)	+	Johns-Finks-Paul (813)			++	Johns-Finks-Paul (813)
Cocoanut oil			+?	Jansen (814)			+++	Jansen (822)
Cocoanut margarine			?	Pickard (815)				
Brazil nut	++	Cajori (816)	+?	Coward-Drummond (605)				
Barcelona nut	++	Cajori (816)	+?	Coward-Drummond (605)				
English walnut	++	Cajori (816)	+?	Coward-Drummond (605)			++	Cajori (816)
Black walnut	++	Cajori (816)						
Almond	++	Cajori (816)	+?	Coward-Drummond (605)			++	Cajori (816)
Almond oil			0	Osborne-Mendel (570)				
Butternut			+?	Coward-Drummond (605)				
Chestnut	++	Cajori (816)						
Pecan	++	Cajori (816)						
Pine nut	++	Cajori (816)					++	Cajori (816)
Filbert nut	++	Cajori (816)					++	Cajori (816)

PRODUCTS	B-VITAMINE	LITERATURE	A-VITAMINE	LITERATURE	C-VITAMINE	LITERATURE	PROTEIN	LITERATURE
			Miscellaneous					
Malt	++	van der Wielen (823)			++	McClendon-Cole (828)		
Malt, soup					+	Gerstenberger (829)		
Beer	0	Harden-Zilva (824)			0	Harden-Zilva (824)		
Palm oil			++	Drummond-Coward (592)				
Olive oil			0	Schabad-Sorochowetz (38)				
			+	Drummond-Coward (592)				
Flower nectar	+	Dutcher (825)						
Pollen	++	Dutcher (825)						
Coffee beans, roasted	+	Mattei (697)						
Yeast (beer)	++++	Schauman (2)	?	Osborne-Mendel (600)	0	Hess (792)	+++	Funk (325); Osborne-Mendel (338)
Yeast, heated	+++	Karr (404)						
Yeast, autoclaved	++	Karr (404)						
Yeast, baker's	+	Karr (404)						
			Animal Products					
Milk, raw	+	Chick-Hume (826)			+	Chick-Hume (756)	++++	Osborne-Mendel (72)
	+	Osborne-Mendel (326)						
Milk, skim					+	Barnes-Hume (764)		
Milk, skim, dried			+	Coutts (772)	+	Hess-Unger (631)		
					+	Winfield (768)		
Milk, stored					Trace	Hess-Unger (758)		
Milk, cooked slightly					+	Barnes-Hume (764)		
Milk, pasteurzed					Loss	Hess (757)		
Milk, condensed	?	Sekine (771)			+	Hart-Steenbock-Smith (765)		
Milk, sterilized	?	Gibson-Conception (755)						
Milk, summer					++	Barnes-Hume (764)		
Milk, winter					+	Barnes-Hume (764)		
Milk, protein free	++	Osborne-Mendel (72)						
Casein, commercial	Trace	Funk-Macallum (86)	Trace	Drummond (604)			++++	Osborne-Mendel (72)
Butter	0	Funk-Macallum (86)	+++	McCollum-Davis (75)				

Butter oil			++++	Osborne-Mendel (77)				
Lactose	+	Drummond (313)			0	Cohen-Mendel (374)		
Cheese	0	Cooper (643)						
Horlick's malted milk							++	Wheeler-Biester (786)
Nestle's food							++	Wheeler-Biester (786)
Egg powder, whole							+++	Baglioni (720)
Egg, cooked					0	Hess-Unger (375)		
Egg, yolk	++	Cooper (643)	++	Osborne-Mendel (830)				
	++	Steenbock (507)						
Meat, lean, raw					0	Dutcher-Pierson-Biester (783)	++++	Weill-Mouriquand (392)
					+	Parsons (191a)		
Meat, lean, cooked					+	Willcox (782)		
Meat, frozen							++++	Weill-Mouriquand (392)
Meat, dried	+?	Osborne-Mendel (776)			0	Givens-McClugage (784)		
Meat, powdered	++	Cole (778)					++	Cole (778)
Meat, salted							++++	Weill-Mouriquand (392)
Meat, sterilized	+	Weill-Mouriquand-Michel (392)						
Meat, canned	0	Weill-Mouriquand-Michel (392)					+++	Suzuki and co-workers (836)
Meat, juice					0	Dutcher-Pierson-Biester (783)		
Liver (ox)	+++	Sullivan-Voegtlin (506)			+	Parsons (191a)		
	+++	Cooper (643)						
Myocardium (ox)	+++	Cooper (643)						
Striated muscle	++	Cooper (643)						
	+	Osborne-Mendel (777)						
Pig heart	++	Osborne-Mendel (777)	++	Osborne-Mendel (777)				
Pig kidney	++	Osborne-Mendel (777)	++	Osborne-Mendel (777)				
Pig liver	++	Osborne-Mendel (777)	++	Osborne-Mendel (777)				
Spinal cord	++	Voegtlin-Towles (505)						
Spinal cord, hydrolyzed	+++	Voegtlin-Towles (505)						
Pig brain	+++	Osborne-Mendel (777)						
Sheep pancreas	++	Eddy (831)						
Endocrine glands	+++	Swoboda (790)	0	Emmett-Luros (833)				

PRODUCTS	B-VITAMINE	LITERATURE	A-VITAMINE	LITERATURE	C-VITAMINE	LITERATURE	PROTEIN	LITERATURE
Pig fat	0	Sullivan-Voegtlin (506)	+	Daniels-Loughlin (87)				
			0	Drummond-Coward (592)				
Horse fat			++	Drummond-Coward (592)				
Dog fat			++	Drummond-Coward (592)				
Sheep fat			++	Drummond-Coward (592)				
Oleomargarine			+	Halliburton-Drummond (834)				
Beef fat			+	Osborne-Mendel (830)				
Beef fat oil			++	Osborne-Mendel (830)				
Fish	+	Drummond (835)			0	Parsons (191a)	++++	Drummond (835)
Fish oil			+++	Drummond (582)				
Fish oil, reduced			0	Drummond (582)				
Fish oil, oxidized			+++	Drummond (582)				
Codfish	+	Drummond (835)	++	Drummond (835)			++++	Drummond (835)
Codfish testicles			++	McCollum-Davis (837)				
Cod liver oil			++++	Osborne-Mendel (570)	0	Cohen-Mendel (374)		
					0	Hess-Unger (375)		
Herring	+?	Drummond (835)	++	Drummond (835)			++++	Drummond (835)
Sardine	0	Weill-Mouriquand (392)						
Salmon, canned							++++	Drummond (835)
Shark liver			+++	Sekine (832)				
Tuna fish muscle			+++	Sekine (832)				
Tuna fish liver			+++	Sekine (832)				
Shya-chi-no (Japanese fish)			+++	Sekine (832)				
Miscellaneous Animal Products								
Honey	?	Dutcher (825)			0	Faber (827)		
Erepton							+++	Suzuki and co-workers (323)
Amimo acid mixture							0	Suzuki and co-workers 323)

PART III

The Human Avitaminoses—Conditions in which the Vitamines Play a Rôle

CHAPTER I

Beriberi

The first stage of vitamine research, which we shall assign to the period between 1910 and 1920, served as the instructive period. The vitamine hypothesis, which can really no longer be regarded as such, was promulgated during this period, and will survive in a more or less modified form. The stage of enthusiasm, in which all possible pathological conditions were regarded as deficiency diseases, has passed and we shall endeavor now to treat the matter from the objective point of view. In the study of the known and also the suspected avitaminoses, we meet with the real difficulty that most of the medical men are divided into two camps. One champions the vitamines, while the other will have none of them. The resultant feud is not very conducive to a speedy solution of the questions that interest us here, for, if one has a preformed idea, then all facts are viewed in a light best in accord with this idea. We find, in addition, little coöperation between experimental investigators and clinicians. This would be the more desirable, because most investigators have very little clinical experience, while the clinician lacks the broad scientific basis of the modes of nutrition.

We frequently read in clinical papers, in connection with the description of a possible avitaminosis, that dietary changes and vitamine therapy have been resorted to without success. This whole situation is usually disposed of in a few words and there is frequently a failure to report important observations. In purely scientific investigations, as in the field of physiological chemistry, physiology or pharmacology, it remains for the author of the particular subject to prove his results. Very frequently, his facts interest us far more than his conclusions. In the clinical work on avitaminoses, we often see the conclusions of the individual authors merely as personal impressions, without giving all the particulars, upon which basis, perhaps, a different conclusion might be possible. In the interest of progress, it would be very desirable if in the clinical investigations in this field, an exact description of the previous diets, as well as of the vitamine therapy, were given. In this way, there would be the opportunity, in every individual instance, of forming a

personal conception of the cases quoted, which is now usually quite impossible. Since our first edition, no great progress has been made because of the above reasons, in spite of many publications. The conditions regarded at that time as avitaminoses are considered as such to the present day, while the suspected avitaminoses are still as uncertain as before. The author, as the originator of this classification would like to see all these conditions demonstrated as avitaminoses; on the other hand, it would be worth while to eliminate as soon as possible all that does not belong to this complex.

Even as regards the definite avitaminoses, we are well aware that there will always remain a minority who will have a different opinion from ours. While we recorded all of these divergent opinions in our first edition, we believed it necessary, from the point of view of uniformity of the subject, to avoid this at present. It should, however, not be thought that we wish to ignore the opponents of the vitamine subject. This book is intended to treat of vitamines and we shall not stray from its main purpose.

What has just been said applies even more to the description of those pathological conditions which cannot possibly be regarded as avitaminoses, but in which the presence or absence of vitamines may play a part. The consideration of these conditions in this book should, however, not be construed to indicate the author's belief that they may be related to the vitamines directly. It is possible that some of the diseases to be described here may in the future be explained differently. This book merely pictures the possible aspects of future vitamine study, and does not exclude another solution of the problems.

The reader may ask why an answer to the above problems is not yet at hand. Why can we not say whether or not a certain condition can be associated with the vitamines etiologically? Apart from the fact that the vitamines are indisputably essential for life, we know very little regarding their physiological significance. To be sure, we see signs of a generally diminished assimilative ability, negative balances of some important inorganic and organic constituents, and decreased resistance to infection. Besides this, we see changes in most organs, in the glands of internal and external secretion, and trophic changes in the skin. But just how all these conditions can be correlated with the lack of vitamines is at present unknown to us. The above list of symptoms is so large that we

may associate them with all possible conditions. If now we consider that there is the possibility that new vitamines may yet be found, we recognize the many-sidedness of the possible vitamine aspects.

The investigation of the avitaminoses is rendered more difficult, in that the symptoms characteristic of these conditions may be attributed to entirely different causes, aside from the deficiency of vitamines. Thus, we see the development of edema, resembling very much the hunger edema, which is, however, not of dietetic but of infectious origin. We note beriberi-like symptoms, not related to lack of vitamine B but to toxins and poisons; this is also true of many trophic appearances. Because of these complications, the literature on deficiency diseases has become very clouded, since it frequently happens that the finding of a case explainable by something other than vitamine deficiency is often followed by the attempt to discredit the vitamines entirely. For these reasons, we must be careful in judging the etiology of sporadic and isolated cases; it may easily be associated with an error in diagnosis. If such cases occur in great number (as is usually found in practice) then a mistaken diagnosis can scarcely be made.

The vitamines have lately been associated with orthopedic surgery, certain eye troubles, such as hemeralopia and ophthalmia; some infections such as tuberculosis, pneumonia, leprosy; some metabolic diseases such as arthritis, diabetes (also cancer); and with dental caries. We know now that these conditions have some relation to the diet but whether they are specifically associated with vitamines has not yet been shown, and is in fact difficult to do so. Since many of these pathological conditions, in spite of tiresome research, have given no real explanation, we see no harm if vitamine research will give a new impulse to the study of these questions. We see many examples of how a misleading hypothesis has led to a favorable solution of some questions; at any rate, if a subject remains quiescent it can never lead to progress.

In the description of beriberi, we shall dwell exclusively on the human type, since many phases of this question have already been treated in avian beriberi. Above all, we recommend to the reader who is particularly interested in beriberi the fundamental work of Vedder (838) as well as to contributions by Castellani and Chalmers (839) and Schilling (840), who treat the subject from the

viewpoint of the modern conception of beriberi. Beriberi is a disease which, according to Findlay (841), has been known for hundreds, if not for thousands, of years. Its occurrence was described in the Roman army which invaded Arabia in 24 B.C. Chinese writers in the second century referred to this point. *Neiching*, the oldest medical book (2697 B.C.), speaks definitely of this disease.

East Asia, including the group of Polynesian Islands, is the main beriberi zone. The second greatest area is in Brazil and the neighboring countries. Furthermore, the African coast shows numerous foci. It was commonly held that a moist warm climate was especially conducive for the development of the disease This report is incorrect as, for example, in the Russo-Japanese War, a severe outbreak of beriberi was noted in the Japanese army in spite of the severe winter; beriberi is endemic also in northern Japan. The above mentioned statement is based largely upon the fact that rice grows well only in moist heat. Japan is the most afflicted country. Bälz and Miura (842) estimated the number of beriberi patients at about 50,000 yearly. China is very much less afflicted, though in Korea we find numerous foci. Furthermore, we find the disease quite well spread over the Malay Peninsula. The number of cases there was estimated by Fraser (843) in 1911 to be 5540, among which 695 deaths occurred. This is true also of the islands of the Malay Archipelago, Java, Borneo, Celebes, the Molucca Islands, New Guinea, and especially Sumatra. Simpson (844) reported cases in Singapore. where the disease was often noted among Chinese coolies. In Hawaii, New Caledonia and North Australia, the disease is mostly seen in Japanese and Chinese immigrants. More beriberi zones are found in Cochin-China, Siam (according to Hepburn, 845) and Burma, at the mouth of the Ganges (according to Mulvany, 846), on the east coast of the East Indies, Ceylon, Malabar, and less so on the Koromandel Coast.

In Africa, numerous foci exist on the east and west coasts. Thus, Chevalier (847) reported on an outbreak in Serenli (East Africa) with 112 cases in six months, with 44 deaths. In the Congo, the rice eaters are often attacked by the disease, while other natives, to whom rice feeding is unknown, remain free from the disease. Dubois and Corin (848) describe a number of cases in Bokala (Belgian Congo) which were caused by the consumption of "maniok." Similarly, beriberi is known in Madagascar, and in the Mauritius and Réunion Islands.

The Philippines and Brazil form two important foci. From Brazil, there is a report of Lovelace (849), who observed 934 cases within four years; the disease is known there for 250 years. The beriberi zone broadens out to the north, till Rio de la Plata. Lovelace is not the only observer in Brazil; we have also the reports of Wolcott (850) and Fraga (851), the latter having seen cases in Bahia. Riddel, Smith and Igravidez (852) described cases in the military hospital at Porto Rico. From the previous chapter, we saw that it did not require a consumption of white rice to produce the disease. Little (853) saw a number of cases in Newfoundland resulting from the ingestion of white flour. In the United States, cases of beriberi are very rare occurrences. Parker (854) describes an outbreak of this disease in the prison at Elizabeth, N. J., as a result of eating white bread. Reed (855) saw cases in California among Chinese and Japanese. Travis (856) described cases in Eddyville, Kentucky.

In Europe, beriberi was observed in lunatic asylums in Ireland in 1894, 1896 and 1897; in 1894, there were 147 cases in Richmond Asylum near Dublin, with 25 deaths. Chantemesse and Ramond (857) reported an outbreak of beriberi in the lunatic asylum in Saint-Lemmes (Angers), in 1898, resulting in 40 deaths. Schüffner (858) believed, in general, that beriberi cases occurred sporadically from time to time and were treated under another name. Recently, cases were observed by Martinez (859) in Spain and Portugal. We recognize from this survey that beriberi occurs in places, such as prisons, hospitals, etc., where the food is prepared in quantities in large kettles. A further opportunity for the occurrence of beriberi is provided by wars. In the Russo-Japanese war, the Japanese, according to Schilling (l.c. 840), had about 70,000 to 80,000 cases, while the Russians, as we shall see later, had only scurvy cases. In the last war, we have similar observations. Sicard, Roger and Rimbaud (860), Roger (861) and Leggate (862) reported on numerous cases among Chinese and Indo-Chinese laborers, who were transported to France for labor during the war. Massalongo (863) described cases on the Italian front. Willcox (864), from the Mediterranean area in the English army in 1916, and later also from Mesopotamia (865), reported cases which could not be attributed to rice consumption. Braddon (866) investigated a number of cases in the Mediterranean war zone. Hehir (867) and Sprawson (868) observed cases during the siege of Kut-el-Amara.

MODE OF OCCURRENCE OF AND DIETS LEADING TO BERIBERI

It is very important for the proper understanding of the nature of beriberi to know the composition of the diet leading to its occurrence. This is the more important since a number of investigators have reached the conclusion that the diet of their patients was completely satisfactory, from the viewpoint of nutritional studies. As for this, we may bring to our support the slight amount of exact data available from two sources. On the one hand, we know the composition of the diet in a few instances where the disease has been produced experimentally in man; on the other, we have the reports of a few beriberi outbreaks, in which the composition of the diet was accurately noted.

In the first series, there is the work of Fraser and Stanton (l.c. 47), who produced beriberi in healthy Japanese coolies on a diet made up almost exclusively of white rice. Here, 80 to 90 days passed before the disease made its appearance. Another, and far more important, investigation was that of Strong and Crowell (869). They conducted experiments with volunteers from a prison in the Philippines. The subjects were divided into three groups, each group receiving a certain amount of fish, lard, bananas, potatoes and sugar. Rice, in three different forms, was the chief component of the diet.

GROUP	RICE	NUMBER OF VOLUNTEERS	NUMBER OF BERIBERI CASES
1	White rice and polishings	8	2
2	White rice	17	13
3	Unpolished rice	7	1 (mild form)

From this, it is evident that unpolished rice can practically prevent the occurrence of beriberi, though the vitamine B content of this kind of rice barely exceeds the necessary amount. The first symptoms were noted after 60 days. Caspari and Moszkowski (870) reported an experiment upon Moszkowski himself. He lived 230 days on white rice and other vegetable food, without meat, eggs or cheese, and developed mild beriberi, but with cardiac symptoms and considerable nitrogen loss. On the addition of rice polishings, the symptoms promptly disappeared. It seems likely that that diet contained small amounts of vitamine B, since the development of the disease was so long delayed. Fraga (871) conducted an experiment with nine prisoners. He was unable to produce the disease with

sterilized rice and sterilized beans during a period of 43 days; the subjects objected to the food, and the experiment had to be terminated.

The second group of the accessible data pertains to the composition of the diet leading to beriberi. Chamberlain (872) investigated the personnel of the native Philippine Scouts comprising 5200 men. Of these, 618 developed beriberi in 1908, and 558 in 1909. The food leading to this outbreak consisted of 340 grams fresh beef or its equivalent of bacon, canned meat or fish, 560 grams white rice, 225 grams flour or bread and 225 grams potatoes or onions. When the diet was improved in 1910 by the addition of more beans and unpolished rice, the number of cases decreased to 6. The cases described by Riddel, Smith and Igravidez (l.c. 852), in Porto Rico, developed after three months on a diet consisting of rice, vegetables and canned meat. From the report of Hehir (l.c. 867), we see that there were numerous cases of beriberi among the English troops at Kut-el-Amara on a diet of horse-meat, canned meat and white flour. The siege lasted over $4\frac{1}{2}$ months, and this time sufficed for the development of the disease.

In confirmation of the author's findings (l.c. 559) and those of Braddon and Cooper (l.c. 561) on pigeons, Braddon (l.c. 866) made some observations in the Mediterranean war zone on the dependence of beriberi on the composition of the food. This investigator drew up a table, which we shall reproduce, giving the composition of two diets capable of producing beriberi, compared with two other diets which did not result in beriberi. The content of vitamine B was calculated on the basis of 100 for wheat germ. The quotient $\frac{V}{X}$ shows the relationship between foodstuffs containing and those lacking vitamine B.

This method of calculation is obviously not very exact, but it gives us an insight into the relationship between protein and carbohydrates; in beriberi-producing diets the quotient was much smaller. It is also interesting to note that Braddon, although the individuals were still in good condition, suspected beriberi, because of the lack of reflexes, a suspicion which was later confirmed This showed definitely that beriberi may be recognized prior to the appearance of characteristic symptoms.

FOODSTUFF	VITAMINE VALUE	BERIBERI-PRODUCING DIETS		SATISFACTORY DIETS	
		*Diet A_1	Diet A_2	Diet B	Diet C
Rice		800		600	800
White bread		6400	6400	3200	4800
Jam				200	200
Sugar		400	400	200	
Cheese		400	400	400	
Dried fruit				400	
Salt fish				575	
Margarine, butter or oil		400	400	200	100
X = Total quantity of food lacking in vitamine B		8400	7600	5750	5875
Oatmeal	10			400	400
Fresh meat or bacon	10	1200	1200	850	1200
Peas, beans or lintels	50		800	400	600
Potatoes or fresh vegetables	5	400	400	1200	800
V = Total wgt. of foodstuffs containing vitamine B		1600	2400	2850	3000
Ratio $= \frac{V}{X}$		0.2	0.3	0.5	0.5

* Weekly amount of food.

THE SYMPTOMATOLOGY OF BERIBERI

There is no doubt that a latent form of beriberi does exist (Braddon, l.c. 866). Beriberi symptoms are not infrequently noted, acute for the most part, after a trauma or over-exertion. After an operation, severe fatal manifestations occur in persons who, before the operation, showed no symptoms at all. Acute cases of beriberi occur in soldiers after a long march; light abortive cases are very common. The patients complain of a feeling of heaviness in the legs and of unsteadiness in the knees on walking or standing, so that they frequently collapse. This occurs chiefly in summer months. Very often they complain of palpitation of the heart after an exertion, and of tingling and numbness in the skin of the legs. Skin symptoms in the form of erythema, petechiae, and exanthema are noted. Hemeralopia belongs to the earlier symptoms; the old therapy (liver) of Hippocrates attains thereby a scientific explanation. Hemeralopia belongs also to the symptomatology of scurvy and pellagra, and may

be thought of as a symptom of vitamine hunger. A decrease of sexual ability is frequently observed.

Hepburn (l.c. 845) enumerates, in observations of 100 cases, the following symptoms: The first symptoms, as a rule, consisted in one of three manifestations, (1) edema of the feet and legs in 50 per cent of all cases; (2) numbness, irritability and tingling of the legs in 42 per cent, and (3) epigastric manifestations in 7 per cent. A soft mitral systolic murmur was noted in 20 cases in the first investigation. Cardiac arrhythmia was noted in 5 cases in the first stage. Kato and Yamada (873) noted arrhythmia in the convalescent stage. The body temperature was found subnormal by Doyle (874). As regards the blood picture, Chun (875) found an increase in the number of leucocytes amounting to 100 per cent. The blood pressure was found to range up to 200 mm. by Yoshikawa, Yano and Nemoto (876). Jida (877) investigated the cerebrospinal fluid. In a number of cases he found a large pressure, particularly in the acute stage, up to 280 mm. with a low blood pressure. This fluid, in cases with high pressure, injected into the ear of a rabbit showed a powerful vaso-constrictor action (which indicates, perhaps, a high adrenaline content). Sicard and Roger (878), on the contrary, failed to discover any peculiarities in the cerebrospinal fluid.

The picture of beriberi shows many different forms, according to Bälz and Miura.

1. The light, sensory-motor form.
2. The dry, atrophic form.
3. The wet, atrophic form.
4. The pernicious, acute form.

These forms may also merge into one another. As a rule, the picture of the disease shows paralysis and muscle atrophy in chronic cases; in acute cases, however, there are gastro-intestinal disturbances, anasarca, hydropericardium and severe cardiac symptoms.

1. The sensory-motor form

This is the most common form of beriberi. After an exertion, especially during the summer months, the patient feels unsteady and weak in the legs; often there is numbness of the arches of the feet and of the legs; the calf muscles are sensitive to pressure. In addition, there is palpitation of the heart after exertion, and edema of the

FIG. 55. ATROPHIC BERIBERI (BÄLZ-MIURA)

Fig. 56. Same as Fig. 55, Back View (Bälz-Miura)

FIG. 57. CONTRACTURES OF MUSCLES AND ATROPHY; TIP-TOE POSITION (BÄLZ-MIURA)

legs. The knee reflex is increased at first, and later usually entirely absent; the temperature remains normal. In this stage a cure may result but it may go over into the dry atrophic or the wet atrophic form.

2. The dry atrophic form (figs. 55-56)

In this stage, there is general paralysis and muscle atrophy; at first the legs, then the thighs, hands, arms, and finally the back muscles are involved. The patients then show a skeleton-like emaciation. In this stage, too, improvement may take place under certain conditions, and in the end, after many months, a complete cure may result. The motility, electrical reaction, the original musculature, the patellar reflex—all gradually return. In other cases, reoccurrences appear, the paralysis and atrophy increase, the foot remains in the position of pes equinovarus (Fig. 57); the hands hang weakly with half-closed fingers, as in any radial paralysis. After long prevalence of the disease, permanent contractions of the foot develop, less frequently of the fingers and biceps. The patient can only walk on tip-toes with the help of crutches. These severe chronic conditions are incurable.

3. The wet atrophic form

In this condition, besides the above symptoms, the disturbance of circulation is more sharply prominent. There is palpitation of the heart, tachycardia, dyspnea, oliguria, pain in the calf muscle and edema. Over the ankle there is a pale edema and in severe forms also in the thighs and in the back, especially in the shoulder and neck region; often also in the arms and on the face. Hydropic condition of the serous cavities, namely, the pericardial and the pleural, belongs to the most important symptoms. The elimination of urine is greatly decreased, often to 200 to 300 cc. or even less. The urine has a high specific gravity, is protein free, urates are precipitated out, and it shows a marked indican reaction. If a cure is initiated, the edema disappears with marked diuresis, whereupon the great emaciation and muscle atrophy become apparent. In numerous cases, death follows, partly because of the severe hydropericardium, partly through paralysis of the heart muscle, or, finally, through a merging into the acute pernicious form. In puerperal beriberi, the wet form is noted, as a rule. In severe cases, there is

also paralysis of the intercostal muscle, the diaphragm, and less frequently of the cephalic nerves, optic, abducens, facial and hypo-

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FIG. 58. PARALYTIC FORM OF BERIBERI (FRASER)

glossal nerves. Usually, the laryngeal muscles are paralyzed. Paralysis of the vagus is generally regarded as the cause of death.

Epidemic dropsy. Under this name, Greig (879) described a disease which appeared to result from rice feeding, and which was regarded

at first as identical with wet beriberi. It is likely now, however, that epidemic dropsy, sometimes accompanied by fever, is related not to beriberi but to hunger edema. Greig (880) later investigated the mode of development of the disease somewhat more closely, and found that some natives feed on wheat from which the husk and bran are carefully removed. Addition of meat in these cases exercised a beneficial influence. The question of the etiology of this disease will be taken up again later.

4. The acute pernicious or cardio-vascular form

This variety can occur either primarily or secondarily from the above described forms by over-exertion, after an operation, and also without apparent causes. Quite suddenly, often in but a few hours, a severe picture develops: precordial pains, pain in the epigastrium, dyspnea, tachycardia, nausea, vomiting, diarrhea and staggering. The temperature remains normal and the mind clear. The heart is markedly enlarged, especially the right ventricle; the whole heart region pulsates, due to paresis of the intercostal muscle and the increased heart labor. The visible pulsation in the epigastrium belongs to the early symptoms of the disease. Frequently, there develops a systolic murmur at the apex, and also in the second or third intercostal space; the second heart sound is strengthened. In the arteria cruralis, we hear an arterial-diastolic murmur; in severe cases, even a tone. The pulse is accelerated, 120; in severe cases, 130 and 140, full and soft. The breathing is wild and wheezing, the whole thorax is raised spasmodically. Hoarseness or aphonia frequently develops. The quantity of urine is greatly diminished, 100 to 200 cc. per day, and even less. The urine gives a marked indican reaction; in some cases it contains some albumin, and occasionally it gives a diazo reaction. Death occurs with a small frequent pulse, cyanosis and edema of the lungs, sometimes in but a few days or weeks, but sometimes very suddenly in a few hours.

The mortality in beriberi varies within wide limits. In some outbreaks in Sumatra, Java and Manila, the death rate was frightful—up to 60 to 70 per cent and even higher, while among the Dutch troops in Insulinda 2 to 6 per cent, and among the Japanese troops 2 to 4 per cent. The death rate in the wet form is greater than in the dry, and highest in the acute pernicious form. Formerly, the average death rate was calculated to be 37 per cent, but in recent

years, following a better understanding of the causes and the therapy of the disease, there has been a marked decrease in the rate. A portion of the chronic cases die with complications, chiefly tuberculosis.

FIG. 59. HYDROPIC FORM OF BERIBERI (FRASER)

Beriberi in infants

This form was first described by Hirota (l.c. 743), and develops in infants nursed by mothers suffering from beriberi; usually, the latter exhibit no signs of the disease. The observed symptoms are similar to those of adults: persistent chronic vomiting, cyanosis around the

mouth and nose, dyspnea, tachycardia, aphonia, edema of the face and the legs, oliguria, but no fever. In some of the cases, the mother is still in the latent stage and shows only indicanuria, while more marked symptoms develop later. These statements were confirmed by Andrews (l.c. 744) in the Philippines. He found that a great number of infants died there because of this reason. The milk of such mothers was shown to be poor in fat (2 per cent instead of 4 per cent), while the calcium and phosphorus content was increased. If this milk was given to young dogs, they developed beriberi. The question as to the mode of development and the nature of infantile beriberi was also treated by Lagane (881), Weill and Mouriquand (882) and Walshe (883). In addition, Ogata and Jizuki (884) investigated the influence of beriberi in the mother on the fetus. According to Roxas (885), the disease in the Philippines occurs less frequently in artificially fed children. The great number of cases of infantile beriberi, compared with infrequency of scurvy and pellagra in naturally nourished infants, makes it evident that the formation of vitamine B in the milk proceeds somewhat differently from that of the other vitamines.

PATHOLOGY OF BERIBERI

General

Dürck (886) has written a good monograph on the subject. For the most part, sections were made of acute cases. The skin of the beriberi corpse is quite edematous, cyanotic at the extremities, lips and ears, often with subcutaneous hemorrhages; the musculature is also edematous. One of the most common typical findings is hydropericardium, which develops as a result of the degeneration of the nerves of the pericardium. Hydrothorax, ascites fluid and pinpoint hemorrhages are frequently observed in the serous membranes. In acute cases, ecchymoses are not infrequently noted in the stomach and in the duodenum. The heart, especially the right ventricle and auricle, is markedly dilated and hypertrophied. The heart muscle itself shows yellowish spots and streaks (of a fatty nature). The lungs are usually edematous. The kidneys are full of blood, but here, as in other organs, there are small hemorrhages. Nephritis was not observed.

The most important changes in beriberi were noted in the peripheral nerves and muscles. In the peripheral nerves, no macroscopic changes were apparent, aside from the occasional observation of minute hemorrhages in the sheath or between the fibers. Microscopically, however, all stages of degeneration are noted. The changes begin with the nerve; the medulla becomes varicose, especially near the constrictions; it falls apart in clumps and masses and finally in a granular detritus. The axis cylinder, on the contrary, frequently remains intact for a long time, but in the end this too disintegrates. The Schwann's sheath then becomes a shapeless mass, with increase of the nuclei. In fresh acute cases, the number of degenerated fibers is very slight. At the nerve endings in the muscles, only those nerve fibers leading to the degenerated muscle fibers are swollen and granulated. This degenerative neuritis occurs chiefly in the ulnar, radial and medial nerves in the legs and later in the phrenic and vagus nerves. Bälz (837) found the same to be true of the kidney nerves. Most apparent, however, was the degeneration of almost all of the spinal cord nerves. Bälz (l.c. 887), as well as Ellis (888), found changes in the sympathetic and especially in the cardiac, pulmonary, splanchnic and solar plexi. Rumpf and Luce, (889) found degenerative processes in the dorsal roots. In the brain and spinal cord, as well as in other organs, there is venous stenosis and edematous impregnation. Some investigators have found the motor ganglia cells of the ventral horn atrophically vacuolized. The nucleus lies often asymmetrically, pushed to one side by a large vacuole. Rumpf and Luce found fresh parenchyma and diffused medullary sheath degeneration in the white substance of the spinal cord. The pathological-histological changes in the nervous system will be further described under the subject of pellagra, accompanied by numerous illustrations, for according to the view of an eminent neuro-pathologist, Mott (890), these changes belong, in both diseases, to the same type; however, it should not therefore be said that both diseases must be etiologically related.

The affected muscles show all signs of degeneration and atrophy. The first signs of this consists of the dimming of the striations. Frequently, the muscle fibers become waxy, homogeneous and fragile. For the remaining pathological changes, we may refer to what has been said under the subject of avian beriberi.

Chemical pathology—Blood

The blood-sugar content in 35 cases of infantile beriberi was determined by Suzuki (891) to be 0.0808 per cent. This decrease was attributed to a decreased activity of the adrenals, and is different from that noted in avian beriberi. DeLangen, Schut, Wechuizen and Alting (892) found a very low lipoid and fat content in the blood in beriberi. Arima (893) investigated the non-protein nitrogen in 28 cases and found it increased.

Cerebrospinal fluid

Suzuki (894) found the sugar content of cerebrospinal fluid to be normal.

Urine

Suzuki (895) found the amino-nitrogen of the urine markedly increased in the cardiac form of beriberi. Pagniez and Vallery-Radot (896) studied the elimination of sodium chloride on a diet poor in salt in cases of wet beriberi. The elimination was very large, indicating a previous marked retention. In the wet form of beriberi, as in nephritic edema, sodium chloride passes the kidney only with great difficulty; in beriberi, the water gathers in the muscles, while in nephritic edema, the fluid collects subcutaneously. These investigators believed that the development of wet or dry beriberi is determined by the severity of the kidney lesions.

In confirmation of the adrenaline finding in pigeons, Ono (897) investigated the adrenals microscopically and found a larger quantity of adrenaline, as compared with the amount in other pathological conditions.

THERAPY OF BERIBERI

General

Vitamine therapy in beriberi is at present so well established that we need not dwell on this point unnecessarily. One of the first investigations was that of Thompson and Simpson (l.c. 60), who used yeast for this purpose with good results. The experiments were thereupon extended by Vedder and Williams (l.c. 471) to include a preparation of rice polishings. They thought that they had obtained good results in dry beriberi with a vitamine preparation, made

according to our procedure, while in wet beriberi it was ineffective. From this, it was concluded that for the therapy of various types of beriberi, vitamines of various sources were necessary. This observation may be explained by the severity of the symptoms, without having recourse to such a hypothesis. Good results with vitamine preparations were obtained by Williams and Saleeby (l.c. 472). Later on, Saleeby (898) used autolyzed yeast in adults and children. The dose used was 15 to 40 cc. three times daily for adults, and 2 to 4 cc. every 3 hours for children, greater doses being of no advantage. Marked results were noted after 3 days, but the treatment was continued for 14 days more. In infants, the results were noticeable even sooner. A standardization of the extract of rice polishings was recommended by tel Rosario and Maranon (899). In the late war, a yeast preparation (marmite) was used in the English army with splendid results (Willcox, l.c. 864). Hepburn (l.c. 845) too reported results with the vitamine therapy in Siam.

Therapy in infantile beriberi

This consists either of a change of milk, or of the administration of vitamine. Particularly good results were obtained with an extract of rice polishings (tiki-tiki). According to Albert (900), the symptoms disappear in a few days, sometimes after a few hours. Cox (901) remarked particularly on the decrease in the number of cases of infantile beriberi in the Philippines since the introduction of vitamine therapy.

It need hardly be said that poor results have also been obtained by the use of vitamine therapy. What we said about avian beriberi applies here also, namely, that for a successful vitamine therapy it is necessary throughout that the anatomical changes should not have progressed too far.

SHIP-BERIBERI

It is theoretically quite possible that vitamines B and C should both be lacking in the diet, and in this way give rise to a mixed form of scurvy and beriberi. Such mixed forms have not been described with great certainty, but it would be possible to regard ship-beriberi as such. The various investigators are not agreed as to the nature of this type of beriberi. It occurs on sailing vessels, when the supply

of fresh vegetables approaches the end. Characteristic symptoms are numbness of the extremities, short breathing, and finally death from heart failure. The patients recover very rapidly when they receive fresh provisions, nervous symptoms being noted only seldom; this is not the case in ordinary beriberi. If the crew is made up of representatives of rice-eating people, who keep to their dietary customs, then true rice-beriberi, and not ship-beriberi, develops. Since long sea voyages are now of rare occurrence, ship-beriberi is rarely met with. During the World War, the German raider *Kronprinz Wilhelm*, with a great number of cases of ship-beriberi, was interned in New York harbor. The crew lived on frozen meat, while the officers received a daily portion of fresh vegetables and fruits in addition, and therefore did not contract this disease. Since fruits and vegetables contain both vitamines B and C, the above cases can not give us any clue as to the true nature of the above condition. Nocht (902) was of the opinion that the disease was not identical with rice-beriberi, while Vedder (l.c. 838) believed that it was. Holst and Frölich (903), on the contrary, saw therein a greater resemblance to scurvy, although Holst (904) stated later that on Norwegian ships the crews, which ate rye bread, were free of this disease. However, if the rye was mixed with wheat, the disease developed. From this, it might be concluded that we are dealing with beriberi, in which view Holst was strengthened by the results of his pigeon experiments.

Ship-beriberi is apparently a mixed syndrome, differentiated from scurvy by numbness of the extremities, and from beriberi by scorbutic gum changes.

RELATIONSHIP BETWEEN BERIBERI AND SCURVY

As we have already seen in the previous chapter, we believe that under certain conditions mixed avitaminoses of beriberi and scurvy may arise, though in practice, from a lack of both vitamines, one condition develops in preference to the other. It is nevertheless possible that scurvy should develop on an exclusive diet of rice. During the siege of Paris in 1871, if the observations are correct, such cases occurred and were reported by Delpech (905) and Bucquoy (906). Garcia (907) described a mixed form of scurvy and beriberi. Scherer (908) had the opportunity of observing an outbreak of scurvy in German Southwest Africa, in which there was a death rate of 30 per cent. The disease developed because of an exclusive rice diet

and was accompanied by beriberi symptoms (heart dilatation). In individual cases, anasarca, serous fluids in the pericardium, pleura and peritoneum were noted—symptoms which belong sometimes to both scurvy and beriberi. Greig (l.c. 880) also observed, in cases of epidemic dropsy, scorbutic symptoms, with bleeding of the gums in 10 per cent of the cases. Gouzien (909) described an interesting picture of a disease in Hanoi. At first beriberi developed; if white rice was then replaced by the hand-milled variety, the entire 64 patients promptly recovered, only to develop scurvy somewhat later. The cases of scurvy which develop on decorticated corn in South Africa are explained with some difficulty. Fleming, Macaulay and Clark (910) reported thousands of such cases among miners in South Rhodesia. The composition of the diet there was as follows: Mealie-meal (a corn product poor in vitamine B)—2 pounds daily; meat—1 pound weekly; beans—2 pounds weekly; and nuts (monkey-nuts)—$1\frac{1}{2}$ to 2 pounds weekly. Orenstein (911) is of the opinion that the differential diagnosis of beriberi and scurvy in these cases was not made properly; he could, however, firmly establish the point that these diseases promptly disappear on giving fruit and vegetables. Darling (912) regarded this South African disease as scurvy, whose picture was complicated by beriberi. Among the pathological findings, there was noted hypertrophy of the right heart, fatty degeneration of the heart musculature and the vagus, in addition to accentuated reflexes. In a later contribution, Darling (913) stated that scurvy at the Rand mines can not be so easily cured as pure scurvy, and it was therefore regarded as a mixed form.

What we wish especially to establish by these statements is the fact that we encounter in beriberi, as well as in other human avitaminoses, a pathological condition which perhaps can not be regarded as being entirely due to the lack of a single vitamine.

CHAPTER II

Scurvy

A historical survey of this interesting disease is given by Schröder (914), Schelenz (915), Hirsch (916) and Autran (917); in a new monograph by Hess (918), the whole field of scurvy is critically treated. We have already touched lightly upon the development of this subject and have spoken of the accurate conception of the nature of scurvy by physicians in the eighteenth century—Kramer (l.c. 32), Bachstrom (l.c. 33), Lind (l.c. 34). From all of these data, we see that scurvy has been known for hundreds of years, and makes it appearance particularly on long sea voyages, polar expeditions, and in time of war. Cook (919), in the report of his expedition, mentioned the foodstuffs needed for the prevention of this disease. On one occasion, raw sea-lion meat was used for this purpose; on a second voyage, he used a malt infusion. Based upon these and similar experiences, the English navy in 1804, at the suggestion of Sir Gilbert Blaine (920), introduced the daily portion of lime juice, whereupon, according to Budd (921), the number of cases decreased markedly. It appears certain that scurvy was prevalent in every war, including the last, in great numbers; however, in times of peace it was due to bad harvests, especially of potatoes. MacNab (922) saw numerous cases in the English army at Nassirabad in Rajputana (India) during 1833-1834. The disease was brought to an end by using a native sour plant, *Phyllanthus emblica*. In the Civil War in the United States (923), there occurred 30,714 cases of scurvy, because of the use of dried vegetables in winter. In the Franco-Prussian War of 1870-1871, numerous cases were observed during the siege of Paris, described by Delpech (l.c. 905), Bucquoy l.c. 906) and other physicians (924). The disease was at time time prevalent in prisons and was frequently accompanied by edema. In the Russo-Japanese War, scurvy was observed among the Russians and was described in detail by Sato and Nambu (925) and Blau (926). Sir W. G. MacPherson (927) saw 20,000 cases during the siege of Port Arthur. As regards the disease among the civilian population, it made its appearance when there was a scarcity of potatoes and

fresh vegetables, especially when large amounts of meat were not available. Curran (l.c. 781) reported an outbreak of scurvy in Swift Hospital, Dublin, in 1847, among patients eating almost a pound of meat daily, or drinking a half liter of milk, in addition to their usual food. Rae (928) reported that in the Hudson Bay Expedition, the natives did not develop scurvy because they ate a large amount of meat per day—the men, 8 pounds; the women, 6 pounds, and the children, 2 pounds. Lanceraux (929) reported cases in the prisons of the Department of the Seine arising from a lack of fresh vegetables and potatoes. In Russia, the disease has been prevalent almost at all times, and one of these occurrences has been described by Berthenson (930). Müller (931) observed numerous cases in Nürnberg early in the winter of 1911, arising as a result of a poor harvest. Taussig (932) believed that in Austria, also before the World War, scurvy was not infrequently noted, especially when it was difficult to obtain potatoes. The importance of potatoes as a protective against scurvy is firmly established, and this is also shown by the newest data. In England, especially in 1917, cases of scurvy occurred in poorhouses in Glasgow (Pickens, 933) and in Newcastle (Harlan, 934), which could be attributed to the above cause (lack of potatoes). In a health report of the city of Manchester (935), cases of scurvy were described in the spring of the same year. An article in the *British Medical Journal* (936) at this time showed the serious situation, and the danger of the substitution of potatoes in the diet by rice and bread. The quantity of potatoes necessary to protect against scurvy, according to the Committee of the English Royal Society (937), is about one pound per day. Hess (l.c. 819) showed the etiological relationship between the occurrence of the disease and the amount of potatoes allotted, in one institution where 200 cases were involved. Lind (938) describes a number of cases in a hospital at Kew, Victoria (Australia), while G. R. Hopkins (939) observed 3000 cases in Aruba, a small island in Dutch Guiana, which had a total population of 10,000 in 1915. The cause of the disease was a total crop failure during the period 1912–1914. The diet of the natives consists of corn, corn meal, salt fish and a small amount of meat, mostly dried or salted. The well-to-do class, which imports fresh vegetables and fruits, were free from this disease. With the arrival of the rainy period, and the consequent availability of fresh vegetables, the disease disappeared completely.

During the World War, thousands of cases were observed, and it is most likely that no single army was entirely immune. Hehir (l.c. 867) described numerous cases among the Indian troops, in Mesopotamia, who refused to eat meat because of religious scruples. Turner (940) states that 30 to 50 per cent of these troops developed scurvy, and Wilcox quoted the following figures:

1916	11,445
1917	2,197
1918	826

On the other hand, among the English troops, only sporadic cases were seen. The situation was so serious that a corps of gardeners (941) was sent to Mesopotamia by the English government to plant vegetables of every kind and to distribute seeds which could be sprouted when the need developed. L. Hill (942) described an occurrence of scurvy among English troops in Scotland, attributed to excessive cooking of vegetables. The disease seemed to single out particularly those men who were engaged in hard work.

During the late war, the disease was noted in Russia by Hörschelmann (943) and among the Russian prisoners in Turkestan, by Disqué (944). Börich (945), chief surgeon of a Red Cross Station in Russia, personally observed 1343 cases. Among some troops, 75 per cent of the men were disabled by this disease.

In France, Harvier (946) reported that in 1917 95 per cent of his 800 troops developed scurvy, while at the same time the disease was discovered in other detachments. Benoit (947) saw 63 cases out of a body of 350 men, but only of a transient nature; the patients recovered without special treatment. Mouriquand (948) too spoke of a number of scorbutic cases in the army.

For the Italian army, we have the reports of Ferrari (949), Ramoino (950) Gingui (951), and of Vanutelli (952), who observed 200 cases of infectious purpura with hemorrhagic scurvy. Vallardi (953), in Macedonia, saw cases among Italian soldiers who manifested icterus and enlargement of the glands.

From Germany and Austria, we have many reports of the army medical staff, from which it is clear that many of the cases were diagnosed (954) as purpura or rheumatism. The cases of scurvy during the war were so numerous that special stations were opened. Schreiber (955) observed 30 German prisoners suffering from scurvy,

which was at first diagnosed as purpural rheumatism. Speyer (956) was sent by the German government to Bulgaria to make a study of scurvy. Lobmeyer (957) observed scurvy in the Turkish army, but it is possible that some of these cases were complicated by war edema and beriberi. Other cases of scurvy manifested during the war will be discussed in the course of this chapter. Simultaneously with scurvy in adults, numerous instances of infantile scurvy were noted in Central Europe. However, it is no longer necessary to differentiate between these two types.

MODE OF DEVELOPMENT

It is always instructive and of practical importance to know the exact diets that lead to the respective avitaminoses. With regard to scurvy, such data are at hand. Scherer's report (l.c. 908) treat of more than 862 cases of scurvy in the earlier German colonies, among miners and railroad laborers, of which 289 died. The diet there consisted of a kilo of white rice and 500 grams of meat two times weekly, almost without fruits and vegetables. The first symptoms appeared in 5 to 6 weeks. In the *Bulletin of Tropical Diseases* (958) there is a description of a dietary which led to numerous cases of scurvy in the prison at Burma:

	grams.		*grams.*
Hulled rice	684	Spices	4
Beans	114	Fish paste	14
Vegetables	285	Salt	7

It was shown that an addition of vegetables, milk, meat or fish had little effect on the disease, but an addition of sweet potatoes was effective. Dyke (959) described numerous cases among South African natives, transported to France for war work. The number of patients amounted to 680 out of a total of 1700. The diet consisted of 450 grams frozen or canned meat and 225 grams fresh vegetables. In addition, corn or rice, bread and margarine were provided. Vegetables were cooked for 3 hours, which was obviously partly responsible for the outbreak of the disease; aside from this, Dyke noted that the natives drank 3 liters of beer, called "Kaffir," prepared in Africa from germinated kernels, but in France from ungerminated corn. This beer is supposed to protect the natives from scurvy in their home land. During the war, Comrie (960)

was with the English troops in North Russia; in the prisons there, he observed scurvy. The disease began after 4 to 7 months on a diet consisting of 313 grams flour or zwieback; 250 grams rice, oatmeal, peas or beans; 205 grams frozen or canned meat or salted herring; 50 grams bacon or pork; 7 grams tea; 28 grams sugar; 21 grams salt and 14 grams preserved lime juice. Of the antiscorbutics investigated, the order of activity was as follows: Sour milk, fresh meat, fresh lemon juice, germinated peas, canned fruit and germinated beans. Stevenson (961) believes that in adults the disease develops in 4 to 8 months, and that 200 grams cooked vegetables daily may prevent the occurrence of the disease. According to Chick and Dalyell (962), prolonged cooking of vegetables is frequently responsible for the outbreak of scurvy. Forty such cases were observed in Pirquet's children's clinic in Vienna; these cases developed eight weeks after the fresh vegetables were dealt out, somewhat sparingly. These investigators believed also that rapid growth on a diet very rich in calories is especially conductive to the development of scurvy. During and after the war, a number of cases of scurvy were observed among older children, which was very seldom the case in times of peace. Tobler (963) commented in particular upon this, mentioning more than 200 such cases. Erich Müller (964) describes a number of these cases in the orphan asylum (Frederick the Great) in Rummelsburg near Berlin, which were attributed to the use of dried vegetables. Weill and Dufourt (965) noted it in children between $2\frac{1}{2}$ and 6 years old in a zone formerly occupied by German troops.

Regarding infantile scurvy, "Barlow's disease" (cf. reviews by Morse, 966), this condition occurs almost exclusively in artificially fed children. There are, however, a few investigations, for example, that of Netter (957), who described the disease in breast-fed infants; however, from a critical survey of these cases, Hess (l.c. 918) concluded that these data were uncertain, although theroretically not impossible. The cause of the disease is to be sought in the heating of the milk (pasteurization or sterilization). The feeding of artificial milk products such as condensed, homogenized or preserved milk, or of children's proprietary foods as chief diet (Cheadle and Poynton, 968) brings about the disease. After the introduction of the soxhlet apparatus, the milk was not infrequently warmed for 45 minutes and longer. The first extensive investigation of this disease we owe

to Sir Thomas Barlow (l.c. 35). He concluded that this type is identical with scurvy in adults, as did also Holst and Frölich (l.c. 903) and Looser (969).

Through the work of Neumann (970), we recognized the causative relationship between heating the milk and the appearance of infantile scurvy. He observed the development of scurvy on a milk which was first pasteurized at the dairy farm, and on the next day, before consumption, heated in a soxhlet for 10 to 15 minutes. Neumann's conclusions were confirmed by Heubner (971) and by A. Meyer (972). Brachi and Carr (973), in England, reported some cases of of scurvy on cooked milk. In 1898 the American Pediatric Society (974) issued a report on 356 cases, all on artificial feeding. Up to 1908, Cheadle and Poynton (l.c. 968) collected 80 such cases.

More recently, Hess has occupied himself, together with his co-workers, with the subject of infantile scurvy. Hess and Fish (975) found that scurvy in children may be brought about by feeding milk heated for 30 minutes at 145°F. The condition may be prevented by the addition of antiscorbutics. Sittler (976) stated that milk, warmed for 5 minutes on the water bath, does not cause scurvy. Comby (977) described 41 cases, on proprietary foods, among which there were several sent in with an incorrect diagnosis. Epstein (978) reported a number of cases in Prague during the war, at a time when oranges and fresh fruits were scarce; the milk was evidently heated several times before using.

We must also consider the flour diet as an etiological factor. Bendix (979) described a case, on cow's milk diluted with Kufeke's flour food, which was cured by raw milk. Even if the carbohydrates, as such, do not hasten the symptoms, they act unfavorably because of the milk dilution. Hess and Unger (l.c. 656) have shown that malt soup, prepared with the addition of alkali, is productive of scurvy, while Gerstenberger (l.c. 829), under certain conditions, could effect a cure with it. Such cures, however, may also be attributed to variations in the quality of the milk. Fordyce (980) believes that the disease can be attributed to the use of proprietary foods; he rarely saw the disease in children less than six months and more than one year old. Apart from the facts touched upon here, the points to be considered in infantile scurvy are those that have been discussed in the description of the nutritive value of milk.

SYMPTOMATOLOGY AND PROGRESS OF SCURVY

The primary stage in scurvy is characterized by a peculiar paleness of the skin, apathy, melancholy, muscle weakness and slight dyspnea. The skin becomes yellowish, dry and scaly, and is covered, especially on the lower extremities, with petechiae and larger livid spots, due to subcutaneous hemorrhages. In addition, there are frequently pains in the lumbar region and the legs, often regarded as rheumatic. Changes in the skin are frequently noted; thus Taussig (l.c. 932) reports an exanthema of the legs. Aschoff and Koch (981) describe small hemorrhages near the hair papillae (piqueté scorbutique), and an exanthema localized in certain parts of the body, mostly observed in the scorbutic cases during the war in Rumania and in the Carpathians. Similar skin changes were described by Rheindorf (982). Wiltshire (983) saw among the 3000 cases of scurvy in the Serbian army numerous skin symptoms, which he called "hyperkeratosis," a condition occurring in 87 per cent of his cases; later he noted an exanthema of the petechial type, localized in parts of the body, already mentioned by Aschoff and Koch. Wiltshire also observed conical swellings around the hair follicles. v. Niedner (984) observed that infectious exanthema frequently assumes a hemorrhagic appearance in the presence of scurvy. According to the paper of Sato and Nambu (l.c. 925), the skin, as well as the subcutaneous tissue, is often edematous. According to Wassermann (985), subcutaneous, diffuse, painful swellings appear on the thighs; Speyer (l.c. 956) occasionally noted abcesses on the calves. Swellings in the knee caps also appear. The gums become dark red, soft and swollen, especially around carious teeth, although this characteristic sign may be entirely lacking. Sometimes the gums swell so that the teeth are entirely covered. Zlocisti (986) described an ulcerous, gangrenous stomatitis. Aschoff and Koch noted a falling out of the teeth with resultant hemorrhages.

In later stages, the patients suffer very much from dyspnea and palpitation; they faint easily, and heart weakness progresses. The sufferers becomes markedly emaciated, the muscles become atrophic, and contractions are seen at times. Scherer (l.c. 908) described a tip-toe position, which he believed to be the result of hemorrhage; Aschoff and Koch described a characteristic position which they

called "Seiltaenzerstellung." Richter (987) speaks of tetanus symptoms in scurvy. It is not quite clear whether these symptoms belong to the picture of scurvy or whether caused by complications, for example, with beriberi.

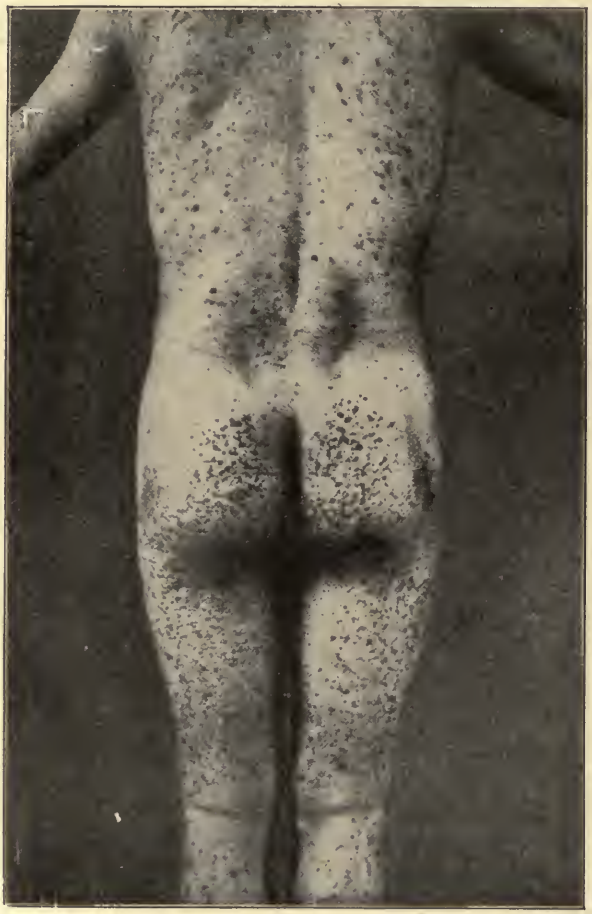

Fig. 60. Skin Exanthema in Scurvy (Aschoff-Koch)

The pulse is rapid; the left and right ventricles are frequently greatly enlarged. Ascites fluid, dystrophic edema, subperiosteal hemorrhages, bloody-serous fluid in the pericardium, pleura, or in any of the large joints—all belong to the picture of scurvy. In

this stage, we meet with persistent diarrhea with bloody-serous stools. Sometimes there is icterus, as was found by Scherer (l.c. 908) and by Urizio (988). Death results with complications of an infectious nature; although it may also be due to heart failure.

Complication with hemeralopia (night blindness) was noted by Zak (989) among Russian prisoners. Hift (990) was able to avoid

FIG. 61. "SEILTÄNZER" POSITION IN SCURVY (ASCHOFF-KOCH)

these complications by means of liver therapy, which shows, perhaps, that the condition of scurvy in these cases was complicated by still another avitaminosis. O'Shea (991) saw 300 cases of hemeralopia among English troops, in 22 cases of which the fundus of the eye was examined. In three of these cases, the only change was a definite paleness. Changes in the retina in scurvy were investigated by Kitamura (992).

Infantile scurvy

Infantile scurvy develops between the 6th and 18th month, seldom earlier or later, and Hess (l.c. 918) differentiated between the acute, sub-acute and latent forms; this view was accepted by Miller (993). The subacute form is not very well defined but growth ceases. Aside from a somewhat abnormal color of the skin and an occasional edema of the upper eyelids, no further pathological signs are noted. The child is capricious, and the lower limbs are somewhat sensitive to touch. The knee reflexes are almost always markedly accentuated. All of these symptoms are promptly overcome on the addition of antiscorbutics, whereupon growth is resumed, according to Hess (l.c. 461).

The latent form is even less pronounced, and the children recover in an extraordinary manner after the administration of antiscorbutics. According to Hess, the latent form occurs especially in cities, where pasteurized milk is sold. It does not need to be particularly exphasized that these less important forms of infantile scurvy occur more frequently than do the acute forms. Franchetti (994) described a chronic case of scurvy in an older child, with bone dystrophy, painful paraplegia, cachexia, anemia, absolute anorexia, but without hemorrhages.

Muscle weakness, anemia, and anorexia, are the initial symptoms noted. Pain develops in the legs; the child holds them motionless and cries out when they are moved. This behavior resembles pseudo paraplegia, according to the statement of Comby (995). Swellings are frequently noted, sometimes only on one extremity, of a periosteal nature, especially of the tibia. Besides this, swelling of the muscles was noted, caused by hemorrhages. The skin on the arches of the feet is frequently edematous, while the skin over the swelling appears pale. If teeth are present, the characteristic gum changes occur. The spongy, bleeding swellings become ulcerous in some cases and the teeth may loosen and fall out. Oliguria, described by Gerstenberger (996), is quite characteristic of this condition, and hematuria less frequently so. The temperature remains normal, occasionally going up to 39°C., following blood resorption after hemorrhages (Barlow fever). When no therapeutic measures are applied, death may ensue as a result of heart failure or hemorrhages. Initial phases of scurvy were described by

Cozzolino (997). He recommended the frequent inspection of the gums for the purpose of the speedy recognition of the disease.

A sort of eczema was noted by Hess (l.c. 918) in 8 cases. In one instance a symmetrical erythema was observed, resembling pellagra erythema very strongly. Proptosis of the eyeball, following hemorrhages, was observed by Cheadle and Poynton (l.c. 968) as well as by Zilva and Still (l.c. 456). It occurs usually in the left eye. Schödel and Naumwerk (998) observed enlargement of the right heart; Hess (999) noted the quite common occurrence of polypnea and tachycardia, apparently because of lesions in the pneumogastricus. Erdheim (1000) also described heart lesions in Barlow's disease.

DIAGNOSIS

From the foregoing, the clinical picture of scurvy is quite obvious and becomes even more simple as a result of the newer possible differential diagnosis, by the administration of vitamine C. Changes in the complexion, follicular changes in the skin, petechiae, subcutaneous hemorrhages, hemeralopia, changes in the gums, feeling of fatigue, shortness of breath, rapid pulse (about 140)—all these are specially characteristic of scurvy. Some times it may be confused with rheumatism and perhaps with hemorrhagic diathesis. In order to differentiate here, the anamnesis is of value as is also the result of vitamine therapy. Occasionally the hematological investigation may be used for the diagnosis, which, however, according to Rosin (1001), shows an unspecific picture of anemia. The so-called "tibialgia" of v. Schrötter was designated as scurvy by Labor (1002).

In the diagnosis of infantile scurvy, the "white line," described by Fränkel (1003) in the X-ray of the bones, has been regarded as characteristic. It is a shadow which is apparent at the epiphyseal ends of the long bones. This indication was observed by Brown (1004) and by many others, although Hess (l.c. 918) did not believe that it was significant in the diagnosis. He observed this shadow also in cases of scurvy, which had been cured for a long time. Hess and Unger (l.c. 759) have introduced a capillary resistance reaction for the diagnosis of scurvy, which consists in the compression of the arm for three minutes with a tourniquet, whereupon petechial spots appear if scurvy is present.

Hematology

A number of investigators have recorded their findings as to the blood picture, but it does not appear that specific indications were discovered. On this subject, there is the work of Labor (1005), Leitner (1006), Brandt (1007), Wassermann (1008) and Benoit (1009); the last investigator reported findings in 63 adults. Hess and Fish (l.c. 975) made hematological studies in Barlow's disease, including the clotting-time of the blood, which was found normal.

As regards the chemistry of the blood, the sugar content was determined in some cases by Rolly and Oppermann (1010) as well as Schumm (1011), and found normal. Hess and Killian (1012) determined the calcium, sugar and urea content, as well as the diastatic and the carbon dioxide combining power of scorbutic blood. They found a moderate acidosis, and a smaller calcium content (different from rickets); as for the rest, the values were normal.

Metabolism experiments

Bauman and Howard (1013) investigated the inorganic metabolism in one case of adult scurvy. The chlorine and sodium balances were negative and were not much more favorably affected by the administration of antiscorbutics. The sulphur metabolism in adults was found abnormal by Labbé, Haguinea and Nepreux (1014). The case investigated was, perhaps, in the convalescent stage and was marked by a large urea output, indicative, possibly, of the disappearance of edema. Experiments in an older child were carried out by Lust (1015), and especially by Lust and Klocman (1016). The child showed a retention of all inorganic constituents, which were subsequently eliminated after a cure; this applies, in particular, to calcium. These data, which seemed to be somewhat uncertain at first, appear now to be corroborated. As for the calcium metabolism, the above results were confirmed by Moll (1017). Frank (1018), experimenting with two cases, found a calcium retention in the acute stage; during convalescence, this calcium was again eliminated.

Pathology of scurvy

A splendid monograph on this subject is that of Aschoff and Koch (l.c. 981) as well as of v. Samson-Himmelstiern (cited by Aschoff and Koch, l.c. 981); with the exception of severe cases, only well

nourished corpses came to necropsy. The subcutaneous tissue, especially of the extremities, is infiltrated with bloody-serous fluid. Widespread blood extravasations, old and new, are noted. The adductor side of the legs shows more hemorrhages than does the abductor; hemorrhages in the muscles themselves are rare. Hemorrhages occur also in the periosteum, especially in the tibia, and in younger individuals in the epiphyseal ends of the long bones and at the cartilage ends of the ribs. Bone investigations in adults were made infrequently but it appears that here, too, fractures are noted. Rosary is often observed, as in rickets. Enlargement of the right and left ventricles, with a possible fatty degeneration of the heart muscles, was also described. Most of the organs were examined microscopically by Aschoff and Koch. In the bones, osteoporosis and a disappearance of osteoblasts were found; nothing of importance was seen in the endocrine glands. Suprarenals showed only a greater lipoid content. Feigenbaum (1019) found hemorrhages in the spinal cord; Scherer (l.c. 908) found hemorrhages in the dura and pia as well as rank growth of connective tissue in the liver.

Ecchymoses occur frequently in the pleura, less often in the peritoneum, and in severe cases in the lungs. Bloody-serous fluids in the pericardium and pleura—less frequently ascites fluids—were found by Johnson Smith (1020).

Barlow's disease

The pathological findings were described by Fränkel (1021), the bones in the infantile form having been frequently investigated. The periosteum is filled with blood and is thickened, but free from small cell infiltration; between the periosteum and the bones widespread hemorrhages are found. At the diaphyseal ends of the long bones, the bone marrow loses its lymphoid character and forms reticular tissue poor in cells and blood vessels which, in stained sections, is characterized by light color, because of "Helles Mark." This degeneration of the bone-marrow is specific for infantile scurvy and prevents normal ossification. The new bone formation is delayed or completely prevented, and the bone tissue present is atrophied, particularly in the ossification zones. In rare cases, hemorrhages are found in the dura, in some joints, in the lungs, spleen and kidneys. As we have already stated in discussing guinea pig scurvy, Hess and

Unger (l.c. 378) showed that a scorbutic rosary may be cured by giving vitamine C. It may seem from this that the complications of scurvy by rachitic signs frequently mentioned in the literature may be attributed to the above condition. By investigation of the bones, however, the scorbutic lesions may be differentiated from the rachitic. In rickets, we find a broad band of osteoid tissue formed at the epiphyseal ends; there is a broad band of insufficiently

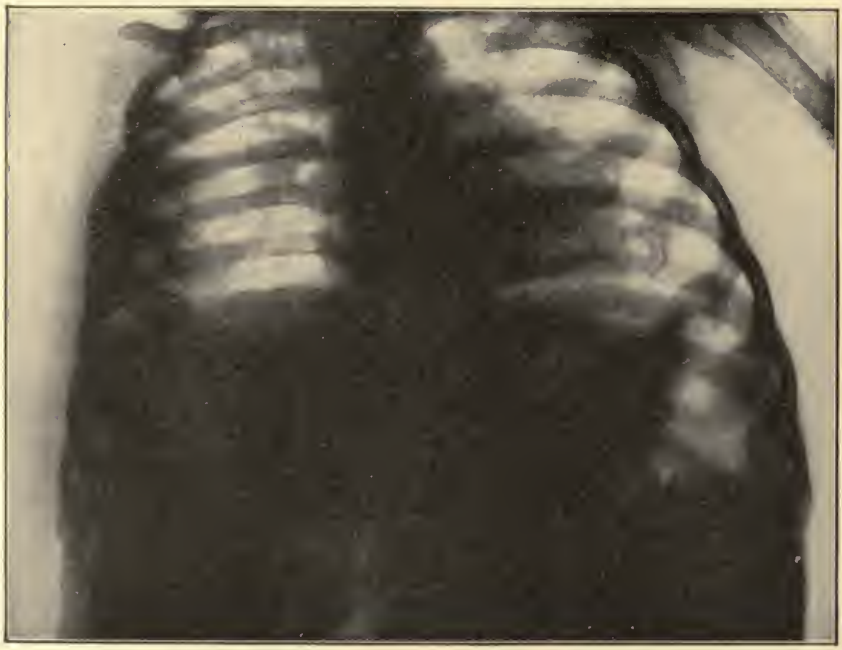

FIG. 62. SCORBUTIC ROSARY

"Scurvy" Hess, courtesy of J. B. Lippincott

calcified cartilage, which is never observed in scurvy. In scurvy, the growth of the osteoblasts is markedly inhibited, but when growth starts, it proceeds in a normal manner. In addition, we find a difference in the number of capillaries in the cartilagenous tissue and in the marrow, very rare in scurvy, but much more frequent in rickets. Hess (1022) saw also a degeneration of the ventral horns in the lumbar region of the spinal cord.

Bahrdt and Edelstein (1023) analyzed the various organs in infantile scurvy. The bone-marrow was poor in solids, calcium and phosphorus. This was also true of the muscles; normal figures were obtained for the other organs.

THERAPY

Since we have already spoken of the antiscorbutic value of various foodstuffs, we need not go further into the matter at this point. On using antiscorbutics, the symptoms disappear, in most cases, after 2 weeks. Even severe symptoms, such as hydropericardium and hydrothorax are very favorably influenced. Still, when the cases have progressed too far, curative results may not be obtained. Paget (1024) treated 133 cases with camel's milk, with good results. As for lemon juice, 24 cc. daily appear to be sufficient for prophylaxis. Germinated peas and beans are also used in practice. They were given to Serbian soldiers, with good results, by Wiltshire (l.c. 806). The treatment of postscorbutic conditions was described by Schulhof (1025).

As for the therapy in Barlow's disease, it consists in the addition of raw or concentrated milk, orange peel extracts, canned tomatoes (Hess and Unger, l.c. 668), and natural fruit juices (Delille, 1026). Hess and Unger (l.c. 637) reported successful intravenous therapy with neutralized orange juice. Harden, Zilva and Still (1027) have used dry lemon juice preparations with great success.

With regard to blood transfusion therapy, Hess (l.c. 918) did not find much vitamine C in blood so that a large amount of blood would have to be used for curative purposes. Similarly, Rueck's (1028) results did not appear any more certain. With a well chosen antiscorbutic, the therapy is almost magical. In 24 to 48 hours, the curative effect is already distinctly obvious.

CHAPTER III

RICKETS

This universally important disease occurs invariably in the first or second year as a result of a deficient diet. In fact, during the last war, the disease occurred almost in all ages, so that the boundary lines between the real rickets, rachitis tarda and osteomalacia have almost disappeared. As we shall see, the inadequate diet of itself, even under the best hygienic conditions, suffices to develop the disease; the rôle of other factors such as lack of air and light, is not ruled out. By and large, the number of cases of rickets increases with the rejection of the natural modes of nutrition and the adoption of artificial feeding of infants. The further child nutrition strays from the normal (breast milk), the less it corresponds to the age of the child, and the more severe are the symptoms of rickets. This disease is chronic and is not dangerous to life of itself, but it does predispose towards infections, especially of the respiratory apparatus.

Rickets occurs everywhere, especially in the temperate zone, less in the north (Iceland, Greenland, Norway), rarer in the tropics and also southern Italy and Spain. Rickets is seldom noted in countries where breast feeding is prevalent, as a rule, but increases in the industrial centers, following artificial infant feeding and lack of sunlight. A racial influence is not noticeable; for example, rickets frequently develops among negroes and southern Italians in America, whereas in their native countries they are unaffected (Cautley, 1029; Strongman and Bowditch, 1029a). The frequently observed racial predisposition may be attributed as well to the dietary customs. Condensed milk together with proprietary foods, as well as a one-sided carbohydrate diet, leads almost invariably to the development of rickets. According to Dennett (1030), there is no danger of rickets from cooked milk, as such. However, even breast-milk may be an insufficient food, especially when the mother's diet is inadequate. We have noted an analogy to this in other avitaminoses, particularly in beriberi.

It is evident that rickets arises when the diet either is lacking in a certain vitamine indispensable for normal metabolism, or contains it in insufficient amounts. We shall speak of other complicating

factors in discussing the etiology of this disease. The missing substance is found in good breast-milk and in cod liver oil, but not in certain milk preparations and flours. It has nothing to do with a lack of fat, protein or calcium, per se, as has been often stated, but with a vitamine which we call "antirachitic vitamine," either belonging to or identical with the type of A-vitamine. In what way the lack of vitamine A brings on all of the known symptoms of rickets, is at present unknown, and we find ourselves upon the same uncertain foundation characteristic of the explanation of all other avitaminoses.

OCCURRENCES

According to the newly found facts, it is unlikely that the disease is associated with any definite age, although it seems certain that it is manifested mostly during the period of active bone growth. Rickets occurs very frequently and we need mention only two investigations to illustrate this. Schmorl (1031) stated that on post-mortem of the cadavers of children under 4 years old, 90 per cent showed signs of rickets. Schwartz (1032) observed among 4944 cases, 15 per cent craniotabes in the first year; 35 per cent showed rosary (of which 13 per cent were in the first month). Congenital rickets too was observed with certainty (Kassowitz, 1032a). Méry and Parturier (1033) saw a case in a 6-weeks old child. Wieland (1034) reported on this too, but he (1035) did not believe in the existence of congenital rickets. Sinclair (1036) and Carr (1036a), held that premature children contract rickets very easily, since the necessary reserve material has not yet been prepared. Hamilton (1037) has described craniotabes in the second month among premature babies. Retterer and Fisch (1038) describe a case of congenital micromelia which they regarded as rickets. Since rickets may occur at any age, the cases of hereditary rickets are not puzzling; the mother may be suffering from a lack of vitamine A during pregnancy, when the requirements are even greater than normally. Among the cases of "osteogenesis imperfecta," there are, to all appearances, those that may be classified as congenital rickets. On the other hand, some of them must be regarded as hereditary osteoporosis, with a poor calcium assimilation. Ostheimer (1039) described a condition in children, called "fragilitas ossium," resembling osteoporosis (lack of calcium), which in this case could be improved not by a calcium or cod

liver oil therapy, but by changing the diet of the mother. He believed that this disease was associated with a disturbance in the metabolism of the mother. One case of osteogenesis imperfecta has been described by Bookman (1040). In this instance, calcium lactate and cod liver oil were used with good results, but it is not quite clear which of the two was responsible for the curative effect. This child progressed even better on breast-milk. Another such case was reported by McClanahan and Willard (1041); the child was born with multiple fractures. Another case was improved with cod liver oil, according to Schabad (1042).

A logical transitional stage is the rickets in breast-fed children. Brade-Birks (1043) reported such a case, and in this respect, Hess and Unger (1044) controlled the diet of negro women in New York in a district where the children suffered markedly from rickets. The diet was recognized as being very inadequate from the dietetic viewpoint.

The deficient nutrition in Europe during the war, especially in the central European countries, brought with it a great increase in the number of cases of rickets, according to Japha (1045). The mortality in rachitic age, due to the pulmonary complications, was greatly increased according to Engel (1046). Weiss (1047) reported that 90 per cent of the children born in Vienna after 1917 suffered from rickets, not excepting the children of well-to-do parents. The disease appeared mostly among children from 1 to 4 years of age. Adams and Hamilton (1048) came to the same conclusion as regards Germany. Rickets in older children was described by Schlee (1049), Stetter (1050) and Sauer (1050a) in Germany, and by Sutton (1051) in Australia; the literature on the subject was reviewed by Frangenheim (1051a). Schlatter's disease, which consists in a separation of the epiphyses of the long bones and other similar fractures, was described in a 13-year old boy by W. Müller (1052) and in a similar case by Paus (1052a); these cases were regarded as late rickets.

The effect of rickets on later life has apparently been underestimated. Apart from the possibility of permanent bone deformity, as reported by Park and Howland (1052b) and by Brusa (1052c), the disease, according to Engel (1053), exerts an influence on the growth of the children, perhaps on the mental development. According to Karger (1054), these children are less active and mentally underdeveloped. In this connection, Léri and Beck (1055) inves-

tigated the French soldiers, and noted that those who had suffered from rickets in childhood showed, aside from anatomical changes, mental deficiency and little resistance to bodily exertion.

SYMPTOMATOLOGY AND DIAGNOSIS

Rickets is primarily a disease of the bones, although it must be regarded as a general disturbance of metabolism. As we gain knowledge of this condition, the latter assertion may perhaps become more justified. The children show a weak muscle tonus and perspire profusely, particularly on the scalp. They commence to walk rather late; the fontanelles remain open longer than usual and the course of the disease may be controlled to a certain extent by measurement of the opening. Swellings of the cartilage ends of the ribs (rosary) are easily palpated, and sometimes are apparent even without this. According to Juaristi (1056) the eyes in rickets are more round and show more of the sclera, associated with bone changes in the fundus of the eye; the penis is long and hangs flaccid. Evidently the delayed development of the teeth is also characteristic of rickets; the enamel is often lacking, and occasionally is covered with a greenish deposit which has been emphasized by a number of authors (1056a). According to Lichtenstein (1056b), rachitic children exhibit an abnormal sense of taste. Various other symptoms associated with slight muscle tonus are described. Characteristic skull formation of rachitic origin is also observed, and as soon as the children commence to walk, the legs bend in the known way, because of the weight of the body.

The diagnosis presents no particular difficulty; the differentiation from scurvy having already been discussed. The differential diagnosis between rickets and some pseudo-rachitic appearances, such as "osteoporosis," is not so easily made. In this respect, the X-ray may be of some help, in addition to the results of vitamine therapy, as compared with calcium therapy.

PATHOLOGICAL ANATOMY AND CHEMICAL PATHOLOGY OF RICKETS

We find, in the work of v. Recklinghausen (1057) a careful and splendid treatise of this subject. The chief sign of rickets is the persistence of the cartilage in the uncalcified condition. The

rachitic bone is recognizable by its abnormally small content of alkaline earths. The deposit of calcium salts is prevented in that part of the skeleton which takes those salts during normal growth; in fact, according to Schmorl (1058), this happens in the entire skeleton. A greatly increased resorption of the already calcified bone is not characteristic for rickets; rather does it characterize pseudo-rickets. In the investigation of rickety bones, the preponderance of the cartilage over the bone tissue is especially marked; for instance, Dibbelt (1059) found, in normal bone, 29.4 per cent cartilage and in rickety bones, 71.3 per cent. According to Schmorl (l.c. 1058), there is an abnormal formation of osteoid tissue. Important changes in the bone-marrow were found by Marfan, Bardouin and Feuillé (1060). Marrow cells were discovered in places where they do not occur normally. These cells were later substituted by fibroid cells with outgrowth of cartilage cells; by this means, the disturbance in the function of the osteoblasts is explained. Hutinel and Tixier (1061) have confirmed the above findings. Kassowitz (1062) found, in rapidly growing bones, an abundance of blood in the epiphyseal ends, causing a proliferation of the cartilage cells and disturbing the normal ossification. Heubner (1063) and Pommer (1064), however, found no inflammation but only a seemingly abnormal proliferation of the cartilage tissue. Ribbert (1065) also noted a marked breaking down of cartilage cells. Besides the bone findings, muscular dystrophy was noted in severe cases by Hagenbach and Burckhardt (1066), Bing (1067) and also by Banu (1067a). According to Mohr (1068), the central nervous system, besides the muscles, also is affected. Of the endocrine glands, du Castel (1069) investigated the thymus and found it hypertrophied. Stölzner (1070) found the suprarenals small with a slight adrenaline content, while Cattaneo (1071) was unable to confirm this.

As for the chemical findings, Dibbelt (l.c. 1059) gave the following composition for normal and rachitic bones:

	NORMAL	RACHITIC
Fat	1.89	7.50
$Ca_3(PO_4)_2$	57.38	15.11
Mg-phosphate	1.72	0.78
$CaCO_3$	8.95	3.15
Other salts	0.83	2.20

Gassmann (1072) found the following:

	NORMAL	RACHITIC
CaO	24.0	21.0
P_2O_5	33.0	30.0
CO_2	3.0	2.75
MgO	0.10	0.53–0.74

In addition, 5 per cent of organic matter was found in rachitic bones. Similar analytical results were obtained by Schabad (1073). Simonini (1074) saw a relationship between the calcium content of the bones and the teeth. Rost (1075) found the water content of rachitic bones higher than in the normal; the ash content of the ribs and the vertebrae, on the contrary, was 20 to 69 per cent lower. Aschenheim and Kaumheimer (1076) found the calcium content of the muscles diminished in severe cases. The calcium content of the blood was found to vary by Aschenheim (1077). Denis and Talbot (1077a) observed a low calcium content in the serum. Howland and Kramer (1077b) frequently found the calcium content of the blood normal, while the phosphorus content was always low. The sugar content of the cerebrospinal fluid was found diminished by Suzuki (l.c. 894).

METABOLISM

In rickets, according to Dibbelt (1078), the elimination of salts in the feces is increased, but decreased in the urine, and sometimes entirely absent. If a cure is brought about, there is first a hyperretention of calcium salts with simultaneous increased elimination in the urine. Since, in his time, the etiology was associated with the amount of calcium in the food, Denton (1079) tried to determine the calcium requirement in children. Aron (1080) tried to prove that milk, especially mother's milk, contains just sufficient calcium to cover the minimal needs. The analyses of mother's milk made by Schabad (1081) were for the same purpose. Orgler (1082) disputed the results of Aron, and attributed them to a faulty calculation of the calcium requirement. Cronheim and Erich Müller (1083) compared the mineral metabolism of normal and rachitic children, and found no marked variations. In Aron's cases, the characteristic metabolic disturbance had perhaps passed. While the assumption of the lack

of calcium in the food could not be confirmed in general, the lesser utilization of calcium in rachitic children has become a fact which has gained support from the numerous reports of Schabad (1084) and others. Schabad (1085) also studied the phosphorus metabolism in various stages of rickets and convalescence. The children investigated were of different ages, both breast and cow's milk being used; normal children were studied as controls. The following figures were obtained:

DIET	CONDITION	P_2O_5 EXCRETION PER KILO PER DAY		RELATIVE P_2O_5 EXCRETION IN PER CENT OF AMOUNT GIVEN		PARTITION OF P_2O_5 EXCRETION		EXCESS IN FECES IN RELATION TO $Ca_3(PO_4)_2$	
		Total	Urine	Total	In urine	Urine	Feces	CaO	P_2O_5
		gram	*gram*						
Breast-milk	Normal 4-5 months	0.023	.0018	65.3	52.8	80.6	19.4	64.8	
	Progr. rickets, 5-13 months	0.034	0.021	122.2	72.2	60.8	39.2	52.8	
Cow's milk	Normal 3-6months	0.214	0.119	70.7	45.1	65.2	34.8	77.8	
	Progr. rickets, 5-8½ months	0.186	0.077	94.6	39.3	39.3	60.7	14.1	
Mixed diet	Normal, 4-5 years	0.15	0.102	80.3	51.5	64.4	35.6	22.0	
	Progr. rickets, 4 years	0.091	0.04	102.1	44.9	44.1	55.9		39.8
Developed rickets 1 year, 5 months to 2 years, 7 months		0.178	0.069	71.8	27.5	38.2	61.8	(16.5)	23.3
Convalescent 2½ to 8 years		0.103	0.077	65.5	48.7	74.4	25.6	35.6	(64)

Very recently, an important series of metabolism experiments in rickets was reported by Schloss (1085a) and also by Freise and Rupprecht (1085b).

According to Flamini (1086) the urinary CaO excretion in normal children is 0.125 gram per day; in rickets, 0.05 gram on an intake of 3.4 grams per day. Peiser (1087) found the calcium balance negative. The nitrogen and sulphur metabolism was studied in rachitic dwarfs by Schwarz (1088), and the mineral metabolism in late rickets, by

Schabad (1089). Schabad's findings on the deficient resorption of calcium were corroborated by Dibbelt (1090).

The study of the influence of the composition of the diet on the utilization of calcium gives some interesting results. The effect of protein was investigated by L. F. Meyer (1091) and by Tada (1092). They found that the nitrogen elimination did not go parallel with the calcium output. The influence of fats was studied by Meyer (l.c. 1091), Rothberg (1093) and Orgler (1094). They found, surprisingly, that the addition of fats (in the form of whole milk) acted unfavorably on the calcium utilization, losses occurring in the feces in the form of soaps. The following figures were obtained on comparing the effect of cod liver oil and butter on the calcium balance:

	WITHOUT COD LIVER OIL	WITH COD LIVER OIL		INSUFFICIENT BUTTER FAT	EXCESS BUTTER FAT
	gram	*gram*		*gram*	*gram*
1	+0.060	+0.175	6	+0.137	−0.198
		+0.141	7	+0.038	−0.034
2	−0.014	+0.143	8	+0.043	−0.120
		+0.519	9	+0.037	−0.267
3	+0.073	+0.303			
4	−0.038	−0.285			
		+0.141			
		+0.108			
5	+0.067	+0.465			

Hess (1094a) demonstrated the unfavorable influence of butter. On the other hand, Telfer (1094b) found that fat, whether in the form of cod liver oil or butter, has no influence on the calcium metabolism. It should be noted, however, that the duration of his experiment was too short (4–5 days); besides, the child under investigation was not rachitic. Hutchinson (1095) found that the soap elimination in rachitic stools was somewhat decreased, 2.2 grams instead of 2.5 grams in normal children. In addition, he (1096) found that the fat loss can be very large in a voluminous stool, giving a negative balance, under certain conditions, of 0.6 gram per day; the fat sometimes constitutes one-third of the total volume of stools.

Holt, Courtney and Fales (1097) demonstrated a large loss of fat in the feces. However, the poor utilization of calcium after the addition of milk fat (butter) could not be corroborated by these investigators (1098).

The addition of carbohydrates appears, according to the prevailing data, to influence the calcium metabolism favorably, although the results were not constant. Reports on this subject were made by Dibbelt (1099), Massaneck (1100), Tada (l.c. 1092) and Rothberg (l.c. 1093). Howland and Marriott (1101) have investigated the action of sugar and cereal addition and found it favorable; but the effect of large excess of carbohydrates was not investigated.

THERAPY AND THERAPEUTIC EFFECT ON THE METABOLISM.

The therapy of rickets with phospho-cod liver oil was introduced by Kassowitz, and is still used almost in the original form. Turning to the findings on the calcium metabolism, the clinical reports on the therapeutic value of cod liver oil are not particularly uniform, and it may be concluded that perhaps not all cod liver oils possess the same therapeutic value. As to this, we have, first of all, the prolific work of Schabad (1102) and his co-workers, in which the favorable effect of cod liver oil is demonstrated; the value of cod liver oil emulsions was also tested. In a later investigation, Schabad (1103) studied the effect of the Czerny-Keller diet on rickets and found that it was evidently not adequate, since the addition of cod liver oil still acted favorably, whereas in a pure milk diet, the oil was unnecessary. Birk (1104) likewise reported on the results of phospho-cod liver oil therapy.

It is only questionable whether in the above combination, the phosphorus addition is necessary. Frank and Schloss (1105) believe that between the two preparations—cod liver oil and phospho-cod liver oil—no far-reaching differences exist; however, Schloss (1106) recommended a simultaneous addition of a calcium preparation. Grosser (1107) investigated the retention of organic calcium salts. Pereida y Elardi (1108) did not believe in the favorable effect of the calcium addition, since a physiologically sufficient amount of calcium is supposed to be present in the diet. Kurt Meyer (1109) studied the effect of cod liver oil therapy on the calcium balance, and obtained good results. Tobler (1110) cured four cases of late rickets with phospho-cod liver oil, and similar results were obtained by Ricklin (1111). Phemister (1112) was of the opinion that phosphorus, as such, possesses no significance in the therapy of rickets, but does stimulate the formation of osteoid tissue. Phemister, Miller and Bonar (1112a) believe now that phosphorus, per se, has a curative action.

However, the number of cases investigated is insufficient, besides which there is the possibility of a spontaneous cure to be considered. As a result of their rat experiments, Sherman and Pappenheimer (1112b) and Pappenheimer, McCann, Zucker and Hess (1112c) are inclined to the belief in the therapeutic influence of phosphorus alone. They believed they could produce rickets in rats on a phosphorus-poor diet, and then effect a cure by the addition of phosphorus. These experiments appeared not very convincing at first, but have since received additional experimental proof. Liénaux and Huynen (1113) do not believe in the therapeutic influence of calcium in rickets.

In opposition to the above more or less definite results, there are the findings of Hess and Unge (1114) on negro children in New York. In these cases, cod liver oil was used without any phosphorus addition. In the negro section, about 90 per cent of all the children are rachitic; the mortality is about 314 per 1000, in which tuberculosis, pneumonia, and whooping cough play the greatest part, it being known that rickets predisposes towards disease of the respiratory organs. Altogether, 40 children were given cod liver oil, while 16 children served as controls. The children were between 4 and 12 months old and the complete results were as follows:

AVERAGE TOTAL QUANTITY OF COD LIVER OIL	TIME OF THERAPY	NUMBER OF CHILDREN	RICKETS	NO RICKETS	NO RICKETS
grams	*months*				*per cent*
1550	6	32	2	30	93
655	6	5	1	4	80
600	4	12	5	7	58
		16	15	1	6

The children were mostly breast-fed and the results of the therapy, especially in cases receiving more cod liver oil, were very marked. These investigators (l.c. 1044) studied also the diet of the mothers and found it very deficient. The results of Ferguson (1115) were about the same as the above. A number of out patient cases of rickets were treated by Mackay (1115a): Small doses of cod liver oil, butter or cottonseed oil had no influence, but larger doses of cod liver oil were effective. According to Aron (1115b) carrot extract was of no value in rickets.

In 1912 Raczynski (1115c) formulated a very interesting theory, quite in accord with the domestication theory of Kassowitz. He thought rickets developed because of a lack of sunlight, a view which seems to have been completely confirmed by the newest experimental investigations. Huldschinsky (1115d) was able to cure rickets by means of alpine light, since the X-ray showed a marked deposition of calcium. Mengert (1115e) used the quartz lamp for prophylactic purposes and Erlacher (1115f) succeeded by this means, in favor-

Fig. 63. X-Ray Diagnosis of Rickets and the Effect of Cod Liver Oil Therapy Seen by this Method

Left, May 22, 1920; right, October 31, 1920 (Hess)

ably influencing 46 cases of rickets. without any change in diet. Hess and Unger (1115g) used sunlight for the same purpose, without any dietary changes, and they (1115h) attributed the seasonal variations in the incidence of rickets to the effect of sunlight. This explanation, among other factors, sounds quite plausible.

In order to follow the course of the therapeutic measures, it is not strictly necessary to carry out metabolism experiments. It may be studied by means of the X-ray, a method utilized by a number of

investigators, among whom should be mentioned Phemister (l.c. 1112), Howland and Park (1116), and Dufour (1116a) and Hess. The results of cod liver oil therapy are apparent in animals sometimes after only two days; in children, after about three weeks, when the deposition of calcium may actually be noted. This method is coming into general use, and will very likely become of great practical importance.

ETIOLOGY

Although we accept the vitamine etiology in rickets, we are well aware that still other views for the explanation of the nature of this disease have been advanced. One of these hypotheses, the causal relationship with the endocrine glands, we have already discussed in detail in our first edition, and will, therefore, not go into it again here. Since the function of the vitamines, especially vitamine A, is entirely unknown, the vitamine hypothesis in rickets does not exclude the possible rôle of the glands in the development of rickets. The study of the etiology of rickets has been in a state of change during the past year. According to the results of modern experimental rickets investigations, this disease seems to be associated with a number of factors a fact which might assign to rickets a special place in pathology. Three such factors have been put in the foreground—lack of vitamine A, lack of phosphorus and lack of sunlight. Each of these factors alone is able to cure the disease, which develops as well in rats and dogs as in man, and has a favorable influence on the calcium metabolism. Similar results have already been obtained with children. Nevertheless, it is not yet clear as to just how these three factors are related to each other. They give, at all events, the same pathological picture. The "domestication theory" of v. Hansemann (1117) and also of Kassowitz (1118) has recently been accentuated by a number of English investigators—Findlay (1119), Paton, Findlay and Watson (1120) and Ferguson (l.c. 1115), and by the modern sunlight hypothesis. Dick (1122) believes that rickets is a disease of great industrial centers, and that it does not occur in the tropics, Japan or China. However, this seems to have little foundation in fact. Although we do not wish to state, at this time, that a poor hygienic condition has nothing whatever to do with the etiology of rickets, we believe, nevertheless, that it can not be the only cause. For, as it most frequently hap-

pens in practice, the unfavorable hygienic conditions are associated with a deficient dietary. Mann (1123) investigated statistically 1000 cases of rickets in London, together with 250 controls. His results speak in favor of the dietetic cause of rickets, inasmuch as he paid particular attention to the mode of nutrition. A critical survey of his cases convinced him that:

44 per cent of the cases suffered from a lack of fat and an excess of carbohydrates in the diet.
16 per cent were breast fed by a poorly nourished mother.
13 per cent occurred in families in straightened circumstances.
6 per cent exhibited a mis-proportion between the dietary constituents, in favor of the carbohydrates.

Czerny (1123a) and Siegert (1123b) allot to heredity an important rôle in the etiology of rickets. That the cause for all rachitic manifestations lies in the central nervous system, was assumed by Pommer (l.c. 1064), and later by Schabad (1124), but this assumption did not exclude the dietetic factor. Other dietetic theories are as follows:

1. Lack of fat and excess of carbohydrates; Herter (1124a); Holt (1125); Cheadle (1126).
2. Lack of a substance of an unknown nature in the diet; Hopkins (l.c. 23).
3. Lack of calcium in the food; Stölzner (1127).
4. Excessively rich food; Esser (1128).
5. Lack of organic phosphorus combinations; Schaumann (l.c. 2).
6. Nutritional disturbances of every description, acidosis and excessive nourishment; Pritchard (1129).
7. Lack of antirachitic vitamine; Casimir Funk; Mellanby.

The vitamine etiology of rickets

This theory was first suggested by us and was attributed to the lack of a specific antirachitic vitamine, in relation to which, the presence of such a vitamine in cod liver oil has been assumed. These conceptions were first tested experimentally by Edward Mellanby (l.c. 95). In the discussion as to the vitamine requirements of dogs, we have already touched upon his work. He divided the foodstuffs used by him into two groups—one, protective against rickets, the other, not.

Not protective	*Protective*
Bread	Whole Milk (500 cc. per day)
Oatmeal	Cod liver oil
Rice	Butter
Skim-milk	Lard
Yeast (10–20 grams per day)	Olive oil
Orange juice (5 cc. per day)	Arachis oil
Linseed oil	Suet
Babassu oil	Cottonseed oil
Hydrogenated fat	Meat
$Ca_2(PO_4)_3$	Meat extracts
NaCl	
Milk protein	

At the time of Mellanby's first publication, the view was largely held that vegetable oils and fats contain no vitamine A and consequently the above table was not so significant as is now the case. In spite of some differences, hard to explain, Mellanby is of the opinion that vitamine A is identical with the antirachitic vitamine. According to his findings, the requirements for this vitamine are particularly large when growth is most rapid. In a later communication, Mellanby (1130) stated that the requirement for vitamine A depends very much upon the composition of the diet. The high protein content of a diet stimulates the total metabolism; the children are active and, therefore, require less of this vitamine. On a carbohydrate diet, the metabolism is sluggish and the organism requires more of this vitamine. However, it seems more logical to regard the action of protein as "vitamine sparing," judging by our personal experience. Besides the composition of the diet, age also plays a big part, according to Mellanby (1131). Rickets is produced in older dogs only with great difficulty, and spontaneous cures often occur. That this is not quite in accord with human pathology is evident in rachitis tarda and osteomalacia. Therefore, the chapter on the etiology of rickets can not, as yet, be regarded as closed. In spite of the fruitful investigations of Mellanby, several points are not yet clear, for example, the prophylactic influence of meat extracts. It is not impossible that Mellanby's experiments have been rendered somewhat obscure through the non-observance of the light factor which will have to be considered in all future research on this subject.

Recently, Noël Paton and Watson (1131a) have subjected the researches of Mellanby on young dogs to experimental testing and

concluded that rickets in dogs is not dependent upon the lack of antirachitic vitamine, but upon the energy content of the diet, exercise and hygiene. However, they admit that dogs on skim-milk develop rickets more readily.

Hess and Unger (l.c. 96), in particular, have subjected Mellanby's results to a sharp scrutiny. They maintained five children, 5 to 12 months old, on a diet composed of 180 grams Krystalak (dried skim-milk with 0.2 per cent fat), 30 grams cane sugar, 15 to 30 grams autolyzed yeast, 15 cc. orange juice and 30 grams cottonseed oil (cereals for older children). Some of these children were kept for several months on this diet, and as they grew older the amount of cereal was increased, no other change being made. At the time of publication, the diet was thought to be completely free of vitamine A. These remarkable experiments showed for the first time that vitamine A as it occurs in milk plays only a minor part in the development of rickets. This finding, which contradicts somewhat the results obtained by Mellanby in his experiments on dogs, was not generally accepted. Hopkins (l.c. 595) held it against Hess' experiments that the above diet was not entirely free from vitamine A, since skim-milk may still contain this substance; cottonseed-oil too is not free from this vitamine. Although these objections are partially justified, we can not doubt that the dietary used by Hess and Unger was indeed very poor in vitamine A. The favorable result obtained with this diet, extremely poor in vitamine A, suggested that the diet per se is not the only causative factor in the etiology of rickets, and that there are still other factors to be taken into consideration. Should they be so interpreted, that rickets has nothing to do with vitamine A, as Hess and Unger naturally concluded at first? On the contrary, these important experiments show that under certain conditions, the vitamine A requirements of children may be very slight, namely, when the composition of the diet as regards protein, salts, vitamines B and C leaves nothing to be desired. The individual differences in children on the same diet may perhaps be attributed to unequal utilization of dietary constituents, a point which has not been allowed for in the experiments of Hess and Unger, or to the influence of light, which was entirely disregarded in all their earlier experiments. In addition Hess and Unger reported, that they observed rickets in children fed on milk (600 to 700 cc. daily) containig 2.5 to 3 per cent fat, and also in breast-fed

children, whose mothers apparently had sufficient vitamine A in the diet. All of these cases were cured with cod-liver oil. Holt, Courtney and Fales (1132) kept a child for 5 weeks on vegetable fat, as the sole fat constituent (apparently free from vitamine A). The child stopped growing but remained in good general condition. Two children developed styes and two others, facial eczema, which disappeared when milk fat was given. v. Gröer (1133) conducted an experiment with two children, who grew almost normally during the first half of the first year on a fat-free diet. The diet consisted of skim-milk, containing only 0.01 per cent fat, and cane sugar; later, oatmeal was added. Here we see again the demonstration that under certain conditions children may thrive with very little A-vitamine.

Hess (l.c. 636), on the other hand, could observe rickets in about 75 per cent of the children on a diet composed of "Eiweiss" milk supplemented with cream, dextrin, malt-soup and orange juice. He admitted, however, that this diet was also poor in vitamine B.

Among the opponents of the vitamine theory of rickets (lack of vitamine A), we find at first McCollum, Simmonds and Parsons (1134), on the basis of their rat experiments, and also Robb (1135). The latter concluded, from guinea pig experiments, that vitamine C affects the mineral metabolism, so that the assumption of the existence of a special antirachitic vitamine does not appear to be necessary. Later, McCollum and co-workers (1135a), Sherman and Pappenheimer (l.c. 1112b and c) and Hess, McCann and Pappenheimer (1135b) conducted some preliminary experiments with rats on diets deficient in vitamine A and in other respects, and produced a rickets-like condition suggestive of a causative relationship of this condition to rickets in man. In 1915, we noted in rats a disease similar to rickets; a scoliosis-like curvature of the spine with rosary develops sometimes in these animals on diets very rich in fat and containing also cod liver oil (l.c. 331 and p. 339). McCollum and his co-workers fed rats on artificial food mixtures and found that a diet poor in vitamine A and phosphorus, but rich in calcium, led to rickets. A simultaneous addition of cod liver oil (but not butter) made the diet satisfactory for the animals. They found that cod liver oil specifically influenced the bone development; the deposition of calcium in the bones could be readily seen in the X-ray in from two to eight days.

Hess, McCann and Pappenheimer (l.c. 1135b) maintain that a lack of vitamine A is not followed by rickets in rats. This is contrary to the findings of Mellanby in dogs, and shows perhaps that butter is very poor in vitamine A [in agreement with the observations of Zilva and Miura (l.c. 574a)] and that dogs and rats show great quantitative differences with respect to their vitamine A requirements necessary to influence bone growth. In this connection, the vitamine A of butter is less resistant than cod liver oil towards oxidation. In confirmation of this, there is the work of Türk (1135c) that melted butter exposed to oxidation does indeed increase the immunity of children towards infections but does not protect against rickets.

In a recent publication, McCollum, Simmonds, Shipley and Park (1135d) have attributed to cod liver oil a specific rôle in rickets. The vitamine contained therein doesn't seem to be identical in its influence with vitamine A, but perhaps it is a question of quantitative relationships. On the addition of cod liver oil the calcium and phosphorus requirements of the animals were markedly reduced. A similar result was obtained on the administration of phosphorus.

On a critical consideration of the vitamine theory of rickets it is apparent that on a certain diet which must be regarded as leading to rickets, not all children develop the disease. On the other hand, a large percentage of the cases may be cured by cod liver oil, but not all cases. Individual differences arise here which can not be explained at present, though we firmly believe that we are on the right track. Recently, of the French investigators, Nathan (1136) has adopted our view.

Undoubtedly, rickets may be brought into causal relationship with vitamine A, although the above-mentioned factors are also of significance. In accord with this, there is the fact that the manifestations of rickets in children, in certain periods of the year, are more marked than in others. Sometimes, as Dr. Hess has told us, spontaneous cures occur in spring. One might be tempted to associate this with seasonal variations in the vitamine content of milk, as has already been partially demonstrated. The influence of light is also to be considered in this respect. Hess and Unger believe in the specific action of cod liver oil, but not of milk fat. We cannot agree with this since it would mean that normal milk contains no substance protective against rickets, and, therefore, most milk-fed children

would suffer from this disease. If we accept the idea, however, that cod liver oil contains the specific substance, then we admit that the diet, which brings about rickets, must be lacking in this substance. But since butter and cod liver oil play the same part in rat feeding, we have to recognize that rickets is caused by a lack of vitamine A. We must accept this logical relationship, if we wish to proceed to the experimental tests of the above conception. In the meantime, it seems to us that the study of rickets is more likely to lead to the desired end if dogs are used in preference to rats, for in the latter only osteoporosis frequently develops. The development of this disease in young dogs shows an appreciable similarity to rickets in small children. With the elucidation of the influence of light, phosphorus and particularly vitamine A, we may hope for a solution of the problem of rickets.

OSTEOMALACIA

Late rickets, which we have already mentioned, forms the natural stepping-stone between rickets in small children and osteomalacia. The latter seems to be due to an identical metabolic disturbance. Some demonstration for the genetic relationship is provided by Ogata (1137). In the province of Toyama in Japan, osteomalacia developed in adults, under conditions apparently similar to those giving rise to rickets in children. Before the war, the disease developed almost exclusively in young pregnant women, in which condition it often assumed a serious aspect. This form of the disease was described by Liesegang (1138) and also by Scipiades (1139), and was mostly associated etiologically with the endocrine glands. A relationship to the pregnancy was often suspected although this condition undoubtedly was only a secondary factor. It is, however, not impossible that an interruption of the pregnancy might be accompanied by an improvement Koltonski (1139a). Januszewska (1140) reported on 3500 cases in Bosnia on an inadequate diet, accompanied by rheumatic manifestations. The cases appeared between December and April and showed a periodicity similar to that already noted in rickets, and which was associated with improvement in the diet. The disease was favorably influenced by cod liver oil.

During the war, central Europe afforded thousands of such cases. This has been observed in Vienna, especially during the summer of

1919 (1141). The frequency of the cases was remarked upon by Partsch (1142) and by Sauer (1143). The disease did not occur only in the adolescent period (12 to 20 years), according to Hamel (1144) and Heyer (1145), but also in men, according to Köpchen (1146) and in old age, among men and women, according to Curschmann (1147).

Symptoms

Chelmonski (1147a) describes two forms of alimentary bone disease which prevailed in Poland in 1917–1918; one form was accompanied by pain in the joints and brittleness of the bones; the other, more like the classical osteomalacia, but in the absence of pregnancy, with severe changes in the pelvis and progressive paralysis of the lower extremities. As in rickets, the bone changes are most prominent; without this, the diagnosis is not easy and the disease is often confused with rheumatism. In women, according to Croftan (1148), fractures appear chiefly in the pelvic region. The condition was described by Looser (1149) in young people of both sexes (17 to 20 years of age). The symptoms consist in an enlargement of the thyroid, fatigue on walking, pains in the knees and ankles. Pain in the bones is one of the earliest symptoms. Alwens (1150) observed 26 cases among women between 19 and 72 years of age, 65 per cent of which occurred during and after the climacteric. The weight of the patients in most cases was about 100 pounds; 15 cases showed curvature of the spinal column, and in 10 cases, spontaneous fractures were noted. Böhme (1151) emphasized, in particular, the complete analogy between rickets and osteomalacia. Imhof (1152) believed that osteomalacia is frequently accompanied by psychoses (dementia praecox). Gould (1153) believed in a complete analogy between osteomalacia and Recklinghausen's disease and noted that besides an affection of the peripheral nerves in the latter, still other factors play a rôle; in his opinion, the observed bone lesions point to this. Cases of Recklinghausen's disease were described also by Weiss (1153a) and Comby (1153b).

Anatomy

Pathological findings were described by Hanau (1154) and by Wild (1155). The latter observed changes in the bones, similar to those in rickets, namely uncalcified osteoid tissue.

Metabolism

Scott (1156) studied the calcium metabolism in the puerperal type of osteomalacia. The amount of calcium in the urine was increased, while during pregnancy and lactation, the output was decreased. The figures were as follows: normal $CaCl_2$ per liter of urine—1.146 grams; in osteomalacia—1.36 grams; in pregnancy and lactation—0.988 gram. The calcium content of the blood is increased in osteomalacia; in pregnancy, no variation from the normal figure was noted; normal—0.45 gram CaO per liter; osteomalacia—0.58 gram. Leo Zunz (1157) conducted metabolism experiments in puerperal osteomalacia. He found a negative calcium balance, with a definite improvement on castration. Elfer and Kappel (1157a) conducted metabolism experiments with and without the addition of extracts of endocrine glands. The calcium and magnesium balances were sometimes negative, while the phosphorus balance remained positive.

Therapy

The cod liver oil therapy has not been used very much, though where it has been used, the reports were favorable. Looser, Curschmann, Köpchen, Hamel and many others, have reported on this subject. In most cases, a change in diet was instituted, together with the therapy.

Etiology

Hutchinson and Patel (1157b) observed numerous cases among Mohammedan women in Bombay, and attributed the cause of the disease not to the diet, early marriage or prolonged lactation, but to the inertia and lack of air—in accord with the domestication theory of rickets. The description of cases among men, in middle and old age, shows that the disease, as such, has nothing to do with the puerperium. The cases in old age, as well as in middle age, show that this rachitic-like condition has nothing in common with growth, contrary to the findings of Mellanby on rickets in young dogs. Recently, the prevalence of this disease in central Europe was coincident with a lack of milk, eggs, cheese and butter. The population lived on cabbage, turnips, some potatoes and meat. There was, therefore, a lack of vitamine A, as in rickets.

CHAPTER IV

Some Nutritional Disturbances in Children—Tetany, Carbohydrate Dystrophy (Mehlnährschaden), Atrophy

In the description of these conditions, we must state, first of all, that under this heading, besides conditions that really are avitaminoses, others have been included which possess, no doubt, a different etiology. Unfortunately, at the present status of our knowledge, this can not be avoided.

tetany (spasmophilia)

Taking this disease as an example, the clinical picture of spasmophilia is not absolutely defined, and Luzzatti (1158) stated correctly that it is difficult to differentiate between tetany and convulsions, especially because of the atypical course of infantile epilepsy. According to Stheehman and Arntzenius (1159), a poverty of calcium may be followed by clinical consequences of various kinds. The diagnosis of spasmophilia may be simplified by testing the electrical irritability, but this test is not absolutely certain. It is now generally assumed that the tetanic conditions in children are brought about by the lack of calcium. That the calcium retention is impaired in such cases, was demonstrated by Cybulski (1160). Theoretically, however, it is possible that the calcium-impoverishment of the body may take place in many ways. It may be associated with a lack of calcium in the diet, a condition which may be remedied by means of calcium therapy. The disease may be related to an affection of the parathyroids, in which event an organo-therapy would be in order. Finally, it may be associated with an impoverishment of the organism in calcium salts, as a result of rachitic conditions. Pepper (1161) correctly called attention to this possibility. In practice, apparently all three causes may be accounted for.

Etiology

The lack of calcium as the sole cause of the disease must be quite rare, since most of the available data do not report favorably on calcium therapy. When calcium therapy is spoken of, as in the

cases of Rohmer and Vonderweidt (1161a) and Scherer (1161b), this therapy was for the most part supplemented by cod liver oil and phosphorus. Thiemich (1162) found the calcium therapy alone without effect, while Petrone and Vitale (1163) found the parathyroid therapy ineffective in their cases, though Pincherle and Maggesi (1163a) reported that their tetany cases showed pathological changes in the endocrine glands. The third possibility, the calcium impoverishment as result of rachitic metabolic disease, is strengthened by the frequent association of this disease with rickets and osteomalacia. Thus, Januszewska (l.c. 1140) found 338 cases of tetany among 3500 cases of osteomalacia. Sauer (l.c. 1143), too, described this relationship and was strengthened in his view by a favorable cod liver oil therapy. Takasu (1164) associated spasmophilia with beriberi. In his cases, there was a possibility of infantile beriberi. Since the rachitic disturbance may occur at any age, the related tetany may appear similarly. Klose (1165) believed that this disease can not occur under the age of 2 months. Blühdorn (1166) on the contrary, found it in children of all ages. Apart from this, it is not totally excluded that certain forms of tetany of pregnancy and lactation are to be attributed to the above causes, although, this was denied by Faas (1167). Tetany seems to be favorably affected by a one-sided flour diet, and occurs rarely in breast-fed infants, according to Guthrie (1168). Lust (1169) stated that tetany occurs only in 2 per cent of breast-fed and in 55.7 per cent of artificially-fed children. According to Bossert and Gralka (1169a) true spasmophilia may be recognized by the fact that it can be influenced by a change in the diet. v. Meysenbug (1170) did not believe that spasmophilia had anything to do with the three known vitamines, still we cannot see that his results justify such far-reaching conclusions. Jeppson and af Klercker (1170a) believe that tetany is not due to the lack of calcium in the diet but to an excess of phosphorus.

Symptoms

The Erb sign (increase of galvanic irritability of the muscles and the motor nerves) and the Chvostek sign [increase of the irritability of the nerves especially the facial nerve or the popliteus (Ibrahim, 1170b) to mechanical stimulus] are of value in the diagnosis of tetany. According to Stheehman and Arntzenius (l.c. 1159), the Chvostek sign is more sensitive. Bossert (1171) occasionally ob-

served edema and carpopedal spasms. The determination of the calcium content of the blood is of help in the diagnosis. Howland and Marriott (1172) found, in normal cases, 10 to 11 mgm. calcium in the blood; in rickets, somewhat less; in tetany an average of 5.6 mgm. and sometimes only 3.5 mgm. They found that the calcium therapy, even though gradual, is effective. Elias and Spiegel (1172a) found the phosphorus content of the blood very high, while Tisdall, Kramer and Howland (1172b) found that the ratio of sodium and potassium to calcium and magnesium is very greatly increased. Stheehman and Arntzenius (1173) found, on the other hand, that the calcium content of the blood is no definite indication of tetany. The calcium in the blood may be high and yet the Erb sign is positive, which may be associated with a lack of calcium in the tissues. v. Meysenbug (1173a) states that the Thiemich sign is not associated with the low calcium content of the blood and that larger doses of calcium in older children do not affect the anodic reaction. The determination of salt metabolism in tetany was made by Fletcher (1174). Liefmann (1175) found the urinary acetone elimination very high in the acute stage, while Sharpe (1176) found an increased guanidine elimination in the feces. Hoobler (1177) stated that the Ca- Mg- and P balances were all negative. Bolten (1177a) found a decreased blood calcium content with an increase in the electrical sensitivity in Fragilitas ossium (Osteopsathyrosis).

Therapy

Calcium therapy was described by Howland and Marriott (l.c. 1101). Thiemich (l.c. 1162) recommended breast feeding and phospho-cod liver oil. Brown and Fletcher (1178) also recommended phospho-cod liver oil. Stheehman (1179) and Brown, MacLachlan and Simpson (1180) have corroborated the cod liver oil therapy. The last named workers found the intravenous injection of calcium lactate without effect if not accompanied by a simultaneous phospho-cod liver oil therapy. The clinical manifestations of tetany disappear in 10 to 17 days while, without the addition of calcium, the action is very much delayed. Unfortunately, we have found no data on cod liver oil therapy alone. Recently, Huldschinsky (1180a) has used in tetany the sun-ray therapy with which he was successful in the treatment of rickets. It appears that the subject requires still further experimental study.

CARBOHYDRATE DYSTROPHY (MEHLNÄHRSCHADEN)

It is evident that under this name conditions are classified, the etiology of which is fundamentally different. In 1905-1910, particularly in Germany, but also in other countries, widespread diseases of children appeared. These arose as the result of proprietary foods fed over a long period of time. Czerny (1181) was first to recognize the dangers of this regime, and called the resultant condition, "Mehlnährschaden." It need hardly be said that the disease occurred only in artificially fed babies. Children who could tolerate cow's milk poorly or not at all, were fed for weeks, more often for months, on various proprietary foods. Recently, Bloch (1181a) also warned against the danger of a carbohydrate-rich diet in infants. At first, this food seems to be well tolerated; the splendid appearance of the child permits of no suspicion that the food in inadequate. Later, however, after a lapse of time which varies within wide limits in different children, a disease develops which, according to Rietschel (1182), occurs in three different forms: pure atrophic (hunger), atrophic-hydremic and hypertonic.

The pure atrophic form occurs in children fed on flour, with the following symptoms. The stools react acid, with considerable slimy substance and fermentation. At the same time, there develops a severe atrophic hypertonicity of the musculature; the muscles are tightly stretched, which simulates a healthy condition. Electrical supersensitivity of the peripheral nerves is frequently noted. Here, there is often a great susceptibility to secondary infections (thrush, aphthae, abscesses of the skin, otitis, phlegmons, and pneumonia). In spite of the severe symptoms, the gastro-intestinal tract, on autopsy, is found almost intact.

The atrophic-hydremic form manifests itself, at the beginning, in edema of the lower extremeties, which, according to Noeggerath (1183) is due to heart weakness; later, the children show a blown-up face, sometimes also scorbutic changes of the gums (Bogen, 1184).

The hypertonic form resembles peripheral nerve affections very much. In this category, we find cases where the legs are drawn up to the abdomen, with rigid spinal column, tetany with increased electrical irritability and carpopedal spasms lasting for weeks. The prognosis is bad and the children die with neuritis (Grüneberg, 1185).

Etiology.

Langstein (1186) believed in the analogy of this disease with beriberi. In one case, he saw such a great hypertonus of the muscles that he thought it might be a case of Little's disease. Some supposed cases of carbohydrate dystrophy may really have been beriberi. According to Hess (l.c. 918), who noted the development of the disease in the presence of sufficient vitamine C, an analogy with scurvy is excluded. On the other hand, according to some recent work, it seems to be similar to hunger edema. L. F. Meyer (1187) and Rietschel (1188) believed this to be true. Following this, Benjamin (1189) reported that in carbohydrate dystrophy, after feeding "Eiweiss" milk there was high nitrogen retention, pointing perhaps to a craving of the organism for protein. At first, the retention amounted to 50 per cent of the intake; later, 15 to 20 per cent. Since the cause of hunger edema has often been regarded as lack of protein, this statement is of interest. However, since a diet composed chiefly of flour may be very poor in vitamines, under certain conditions, the possibility of dealing with a vitamine deficiency can not be excluded. Hess (l.c. 918) also came to this conclusion. At any rate, Keller (1190) is right when he says that an almost exclusive carbohydrate diet may be used only intermittently. However, there seem to be cases in the literature, as that of Steinitz and Weigert (1191), in which, as a result of a poor tolerance for milk, an exclusive carbohydrate diet was given for a long time. Salge (1192) believed that in individual children, there are great differences in the tolerance for starch. He saw a child fed for 2 months on flour exclusively without any untoward results. The pathological conditions arising from a diet rich in starch were studied also by Abt (1193).

ATROPHY

In the English pediatric literature the term "Mehlnährschaden" is rarely encountered; disturbances of this kind are generally classified as "atrophy" (Cautley, 1194). The latter described the symptoms as follows: The disease arises as a result of an inadequate diet, especially one abundant in flour. There is considerable loss of weight, diarrhea and vomiting. These symptoms are frequently accompanied by stomatitis aphthosa, enteritis, bronchitis and furunculosis. In severe cases, he found edema of the skin of the extrem-

ities, later of the face, petechiae and opisthotonos. On post-mortem, edema of the brain membranes, pleural ecchymoses, erosions in the stomach and intestine, and subepithelial hemorrhages were found. In chronic cases, there was fatty degeneration of the liver and kidney epithelium.

Frank and Stolte (1195) analyzed the organs of children who had died of carbohydrate dystrophy, special attention being given to the liver. This was found to be higher in sodium and chlorine and lower in potassium, while fat, ash, magnesium, calcium, phosphorus and sulphur showed no great deviations from the normal. Steinitz and Weigert (l.c. 1191) noticed, in one case, a high fat content of the organs, and more P_2O_5.

Noeggerath (l.c. 1183) and Hohlfeld (1196) recommended the administration of raw milk. Bendix (1197) recommended breast feeding for atrophy.

OTHER NUTRITIVE DISTURBANCES IN CHILDREN

In many well known nutritional disorders of infants, the present pediatric literature gives no clear picture of the etiology. Above all, the individual pictures are not sharply differentiated. Morse and Talbot (1198) have remarked upon the chaotic condition in the present conception of atrophy. It is therefore all the more difficult for one unacquainted with this subject to have a definite conception as regards the nature of this condition. Of the nutritional disorders to be treated here, there is exudative diathesis, atrophy (in the sense of the German pediatric literature), marasmus, milk dystrophy, etc. Many of these conditions are now thought to be due to excess of fat. In children practice, cases are known in which every possible dietary composition has been tested, but without result; there are even children (though seldom) who can no longer tolerate a completely normal breast diet. How far this may be due to organic disturbances, or to a previous unsuitable diet, is not always certain. In some cases, the deficient diet might have caused such great harm, that the child can no longer be helped, even by the best of diets. Marriott (1198a) differentiated two forms of nutritive disturbances in infants, one form arising after severe diarrhoea and another manifested as chronic malnutrition (marasmus).

For some of the above mentioned nutritional disturbances, Péhu (1199) introduced, in France, vegetable soups as a therapeutic

measure. This therapy was used in Germany by Moro (1200), in the form of carrot soup, with good results. A similar therapy was recommended by Stark (1201) in suspected avitaminoses, and recently, there has been a similar report by Aron and Samelson (1202). McClendon and Sedgwick (1202a) used spinach powder in infant nutrition and obtained satisfactory increases in weight. Aron (1202b) made use of the soluble flour obtained by the action of ferments and found that such products were well utilized in the more severe cases.

From time to time, there appear in the literature statements as to a successful vitamine therapy in certain disturbances. Kohlbrugge (1203) successfully treated cholera infantum, a disease generally regarded as an infection or intoxication, with orange juice. Holt, Courtney and Fales (1204) treated a case of intestinal infantilismus with cod liver oil, with good results.

It need hardly be said that in the feeding of children, the same rules are to be observed as have already been formulated for animals. Besides the already known dietary constituents, we must provide for the presence of the three known vitamines. It is also essential that the more important constituents should not occur in the diet in too great a dilution. Some of the recorded therapeutic results with vitamines depend upon the fact that either the certain vitamines, although they were present, were not sufficient, or the relative proportion of the individual food constituents to each other was not quite correctly chosen. Weill and Mouriquand (1205) have pointed out such a possibility; as regards vitamine B in this connection, we have the work of Daniels, Byfield and Loughlin (1206). We may explain, in this way, certain results of vitamine therapy in an insufficiently defined condition, usually called "marasmus" or "malnutrition." Eddy and Roper (1207), and Dubin and Lewi (l.c. 628) obtained marked results in the conditions just mentioned.

As regards atrophy, it is caused by excess of fat, according to the present conception. The results of Friedberg and Noeggerath (1208) are to be regarded in this light; they recommended the use of centrifuged breast milk in this condition. This recalls certain investigations on rats, made by Funk and Dubin (l.c. 331). These animals received a complete synthetic diet, containing more than 50 per cent fat. The young rats were able to live on this diet for 2 to 3 months. In spite of the vitamine content of the diet, the animals

did not grow; they lost about 50 per cent of their weight and their condition was such as is mostly described in atrophic children. The animals showed a fatty degeneration of the liver; in one case, there was edema and scoliosis, with a permanent curvature of the spine. A physiological explanation of this appearance is still to be found. Should it appear, however, that the milk dystrophy and the decomposition of Finkelstein are attributable to the high fat content of the diet, then the influence of fat may be studied experimentally in the above manner.

In infantile atrophy, Salge (1209) found the assimilative capacity satisfactory. The energy and metabolism of these children was studied by Bahrdt and Edelstein (1210). The assimilation of fat was found normal by Hutchinson (l.c. 1096); fat losses are, however, quite high as a result of voluminous stools. The protein content of the blood was found low by Utheim (1211) and he believed that the protein synthesis proceeds abnormally; in other cases, on the contrary, there may have been an excessive carbohydrate nutrition. Mattill, Mayer and Sauer (1212) state that the glucose tolerance of atrophic children is higher than normally. Gladstone (1213) recommended a fruit-juice therapy. Cure with breast feeding, and in one case with cow's milk, was described by Marfan (1213a).

The exudative diathesis of Czerny (1214) is not associated with lack of vitamines, according to Hess (l.c. 918). The condition, however, leads frequently to scurvy, eczema and petechial hemorrhages. According to Czerny, this disease arises because of the harmful effect of the dietary fat. Schippers (1215) found, in these conditions, a low fat content in the blood. Marfan (1215a) regards athrepsia as a deficiency disease but not as an avitaminosis, and he accepts as the cause a lack of specific enzymes in breast milk. Because of the present better understanding of the nutritive requirements of infants, a fundamental repetition of these nutritional problems is greatly to be desired.

CHAPTER V

Nutrition in Man—An Introduction to the Study of Pellagra and Hunger Edema

From what has been brought out till now, it is clear that in nutrition, it is not sufficient that all the known dietary constituents be present; they must also bear the proper relationship to each other. This idea developed chiefly through observations on rats. If these animals are given a qualitatively correct diet (ad libitum), dietary deficiencies develop nevertheless. It would be tempting to assume that these animals need only ingest more food in order to receive all of the constituents in correct amounts. In practice, however, it is evidently not the case. No matter how much food the animal eats, the relationship of the individual components is not altered. This excessive dilution of certain components may appear in human nutrition, and yield a rather unclear etiological picture.

Pellagra, sprue, hunger edema, and perhaps carbohydrate dystrophy in children, may be associated with the above condition. At least two of the diseases mentioned have recently been attributed to the lack of a protein of high biological value. A number of animal proteins are generally regarded as such. Should this conception prove to be correct, then these pathological conditions must be stricken from the list of avitaminoses, which has in fact already been done by some investigators. We feel, however, that these diseases have been cleared up so little, etiologically, that this procedure seems to us altogether unjustified at present. In the last war, nutrition played a greater role than has been generally assumed by the laity. At the time of the war, we saw one immense metabolic experiment, from which a great number of conclusions could have been drawn, if the serious situation had not prohibited a cold-blooded survey. The late pharmacologist, Meltzer, remarked at one time that all scientific data obtained during the war would have to be corroborated after the war. He may have been correct in this statement, though the convincing power of the large involuntary nutritional experiment remains unquestioned, and we may draw a number of conclusions for our purpose. Various problems must be taken into account in this connection, namely:

1. The question of the protein minimum.
2. Comparison of the nutritive value of vegetable and animal protein.
3. The chemical groups in protein responsible for its high biological value.
4. Influence of war food on the health of the people.

Unfortunately, because of lack of space, we can not enter into a detailed discussion of the above questions. Protein has always been given an exceptional place in the system of nutrition and correctly so. Recently, this has been brought out in the work of Gigon (1216) and Grumme (1217). It is quite important to know the physiological protein minimum, and in this respect, there are the views held by two schools—that of Voit-Rubner, which requires a high protein minimum, and that of Chittenden-Hindhede, which is satisfied with a considerably smaller figure. The figures of Chittenden were appreciably reduced by Hindhede (l.c. 732), who showed (1218) in a great number of extended nutrition experiments, that adults could maintain themselves and work normally on a daily quota of 20 grams of assimilated protein and 3900 calories. The investigations were continued for a longer time than usual for experimental purposes and therefore are very convincing. In one instance, the experiment lasted 305 days and the diet consisted of potatoes (cf. p. 255), margarine, and sometimes onions and fruits. Hindhede believed that when the diet contains sufficient calories, one does not need to worry about the protein content, taking care only that the diet should contain sufficient vitamines. The bread experiments of Hindhede (1219) fall in the same category. Of the bread, made from whole wheat, 30 to 35 grams assimilated protein were necessary. The rest of the diet consisted of margarine and fruits, and the longest experiment lasted 242 days. One experiment with coarsely smashed wheat (l.c. 791) lasted 282 days. In some of these investigations it is even noteworthy that the persons submitting to the experiment did not develop scurvy, though it is possible that fruits were used. The minimum protein figure was very low in experiments with barley-water gruel (1220), with the addition of margarine and sugar. Hindhede (1221) also studied the fat minimum in nutrition which is likewise of importance in the determination of the value of war diets. In this case, the experimental diet was composed of potatoes, cabbage, rhubarb and apples, but without addition of fat. The experimental

subjects were in splendid health for 16 months, so that a lack of animal protein and fat can not be regarded as the cause of pellagra and war edema, since they were on this diet much longer than is required for the development of this disease. Bang (1222) believes, however, that Hindhede's experimental subjects are exceptions and hence his results can not be used without further confirmation, especially as regards children and consumptives.

Hindhede assumed that a gram of potato or bread protein when assimilated, corresponds exactly to a gram of meat or milk protein, but this conception has failed to gain recognition up to the present. In a new series of rat experiments, McCollum, Simmonds and Parsons (1222a) observed that the internal organs, especially the kidney, can supplement effectively the diet of plant origin. Neumann (1223) believes that it is possible to live on an exclusive bread diet. Döllner (1224), however, in the description of the etiology of hunger edema, called it "potato sickness." He saw this condition develop on large amounts of potatoes and cereals. Kraszewski (1225) believes that potatoes are poor in calcium and magnesium and hence must be supplemented with bread and vegetables. Unfortunately the individual investigators are not agreed as to the protein minimum. Jansen (1226) figured that for a man weighing 62 kilos, 2000 calories and 60.5 grams protein are necessary; but the diets used by Hindhede were much richer in calories. Sherman (1227) calculated that for an adult weighing 70 kilos, 44.4 grams protein per day are necessary. Taylor (1228), in his studies on the nutrition of English prisoners in Germany, concluded that 70 to 90 grams protein must be fed. Benoit (1229) investigated in Germany, the metabolism of a number of prisoners (Russian officers) and found that they maintained themselves without complaint for 16 months on 48 grams protein and 1700 calories, the protein being partly vegetable and partly animal. Botazzi (1230) was opposed to the introduction into the Italian army, of Chittenden's figures. Kruse and Hintze (1231) accepted three-quarters of Voit's food standards. Praussnitz (1232) also discussed this matter. Stille (1233) supported the views of Hindhede against those of Rubner. Hindhede recorded the diet on which he and his family lived during the war, which consisted of 54.6 grams protein and 2100 calories. Funk, Lyle and McCaskey (1234) reported a 20 day metabolism experiment on four men, receiving yeast, rice and white bread. A nitrogen balance was

almost obtained with 5 to 7 grams nitrogen. The somewhat unsatisfactory results were perhaps due to a partial absence of vitamines (especially during the periods without yeast), or to a relatively low caloric content. This was one of the few metabolism experiments where the effect of such a dietary mixture on the composition of the blood was studied. As to the nutritive value of bakers' yeast, Hawk, Smith and Holder (1235) reported favorably that yeast can replace 30 per cent of the protein of the diet.

After this brief consideration of the protein minimum, we come to the comparison between the nutritive value of vegetable and animal protein. That there is a great difference in favor of the animal protein, is now maintained by many, in opposition to the view held by Hindhede. The present view of Hindhede was advocated many years ago by Rutgers (1236). Boruttau (1237) showed in metabolism experiments, that vegetable proteins are well utilized; he did not believe that the biological value was an indication of the vitamine content, for unpolished rice has a lower biological value than polished rice. Botazzi (1238), on the contrary, believed in the special nutritive value of animal protein and fat. Röse and Berg (1239) proposed a view, approaching ours, in that they assumed that the protein requirements depend on the nature of protein and the composition of the rest of the diet. Milk protein has mostly been given an exceptional place in nutrition. Recently, this was again reported upon by Hart and Steenbock (1240), and we must, therefore, ask ourselves the question, which chemical group in the protein molecule plays such an extraordinary rôle in nutrition.

This problem has been treated experimentally in such great detail, that a book may well be written on the subject. For this reason, we must confine ourselves to the chief results. The biological value of proteins has been determined by Thomas (l.c. 1) on different products and yielded the following figures:

Beef.....104	Rice.....88	Peas.....56
Cow's milk.....100	Potatoes.....79	Wheat flour.....40
Fish..... 95	Casein.....70	Corn flour.....30

This table is based upon the conception of the similarity of animal proteins with body proteins and is due to their relative content of essential amino acids. A number of amino acids have been recognized as such. Still, in the study of the nutritive requirements of the lower organisms (yeast) it has been established that some proteins

contain substances which, after hydrolysis, cannot be isolated and which thus far remain unknown among the cleavage products. McCollum and Davis (l.c. 97) believed that when casein is autoclaved, its nutritive value decreases markedly; but, according to Funk and Macallum (l.c. 98), this deficiency may be overcome by the addition of vitamine-containing products. Yet, it is not impossible that heating may decrease the nutritive value of some proteins. Mueller (l.c. 167a), in this connection, made an interesting observation in the culture of streptococci, namely, that peptone, casein, edestin, in the tryptophane fraction, contains a substance seemingly indispensable for the growth of this microörganism. Mueller was of the opinion, that he was dealing with a new amino acid, in which belief he was strengthened by the fact that reprecipitated casein possessed the same ability. Still, this condition does not militate against the possibility that the substance may be of a vitamine nature, for in the first place, the active substance is so markedly adsorbable that it may be completely removed with animal charcoal; second, if the substance is carried down in the first precipitation of the casein, it would, in all probability, be carried down on reprecipitation. By reprecipitation of the casein, therefore, no separation can be obtained. We saw in the case of other bacteria that the vitamine-like substances there could not be filtered, even through paper, without being adsorbed. Mueller found that wool, silk, gelatin and hydrolyzed yeast do not contain this substance; it was found in the tryptophane fraction; but when the known substances in this fraction were tested for the above action, no result was obtained. If the fraction is precipitated with phosphotungstic acid, the substance disappears from the precipitate as well as from the filtrate. If the fraction is crystallized, the substance remains in the filtrate and appears unstable. de Souza and McCollum (l.c. 529) observed that hydrolyzed meat exerts some action on the growth of yeast. Funk and Dubin (unpublished work) noted that a completely extracted meat (the last extract being no longer active for yeast), when hydrolyzed, shows definite action. The question whether we are dealing here with a vitamine or an unknown amino acid must be left open for the time being, though it seems to us not impossible that the above finding will give a new stimulus to protein research, in that we have a biological reaction that may be of value in a fractionation. In addition, the nutritive value of the proteins may appear to us in a new light, for the experiments show that some

proteins, in a more or less impure condition (for the degree of purity can naturally not be accurately determined), besides the known cleavage products, still contain something that has been unknown to us. It is very tempting to associate this substance with the etiology of pellagra and similar conditions, though we must admit, that the basis for it is at present hardly at hand. This assumption, however, explains some of the established facts about pellagra very well, as we shall presently see.

DIET*	MEAT	SUGAR	STARCH	LARD	SALTS	AUTOLYZED YEAST	ORANGE	AGAR	COD LIVER OIL
	grams	*grams*	*grams*	*grams*	*grams*	*cc.*	*cc.*	*grams*	*cc.*
Meat	49	12	12	12	3	4	3	3	5
Sugar	12	49	12	12	3	4	3	3	5
Starch	12	12	49	12	3	4	3	3	5
Lard	12	12	12	49	3	4	3	3	5

DIET	WEIGHT—FIRST 25 DAYS		WEIGHT—FOLLOWING 55 DAYS		TOTAL WEIGHT INCREASE†
	Increase	Decrease	Increase	Decrease	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Meat	43	—	86	—	129
Sugar	15	—	55	—	70
Sugar and vitamine‡	15	—	145	—	160
Starch	6	—	62	—	68
Starch and vitamine‡	6	—	165	—	171
Lard	—	9	—	8	−17
Lard and vitamine‡	—	9	34	—	25

* The meat, sugar, starch, and lard were tested and found to be free from vitamine B.

† In this instance, the figure represents the increase after 60 days, and is practically the same after 80 days, since most of the animals had already attained full size.

‡ Vitamine given during last 55 days.

The rats on the protein diet did not require the addition of extra vitamine (autolyzed yeast) at all. This may be regarded as the "sparing action of protein on the vitamine requirement." On the other hand, the rats on the fat diet took the extra vitamine with great avidity, but showed only a small advantage over the controls. The replacement of some of the fat by butter was without any significance, no improvement being noted.

On the starch diet, the rats actually needed extra vitamine (about 2 cc. per day) in order to resume growth. This was likewise true of the rats on the sugar diet except that they appeared not to require as much vitamine for growth as those on the starch diet.

That the protein content of a food mixture is of importance, is apparent from the older work of Osborne and Mendel, who determined the protein minima of various products on rats. The experiments, however, were not carried out from the viewpoint of vitamines. In the consideration of avian beriberi we have seen that an excess of carbohydrates in the diet hastens the outbreak of the disease; an excess of proteins, on the contrary, delays the onset. Funk and Dubin (l.c. 331) conducted some experiments with rats, in which it is easier to control the food intake. The addition of vitamine was constant in all diets and the dietary composition varied only in its content of protein, fat, starch and sugar. The composition of the diets and the results obtained are shown on page 345.

In another series of experiments, rats were kept on a high starch (curve VIII, p. 347) and high meat (curve VII, p. 347) diet, without any B-vitamine. The rats on meat lived much longer than those on starch. The relationship may be seen very well in Fig. 64. Similar experiments were carried out by Maignon (1241), though he paid no particular attention to the vitamines. Grafe (1242) likewise made similar experiments on dogs, and Emmett (1243) took up the same question, but his detailed report has not yet appeared. Experiments with food constituents mixed in unusual proportions were performed also by Osborne and Mendel (1243a). Tachau (1244) suspected, several years ago, that the utilization of certain dietary constituents, such as sugar, may depend upon the protein content of the diet. As for the significance of the above data, they show that the protein concentration in a given food mixture is of greatest importance. The dilution of the protein and vitamines by an excessive carbohydrate addition is not without its harmful sequelae. *In the presence of considerable protein, the vitamine requirements of the organism are reduced to a minimum.* We have no tangible means of explaining this, as yet. The protein may act as "vitamine-sparing," so that less vitamine is required in digestion and assimilation, as compared with other dietary constituents. Perhaps the content of protein in the new substance, mentioned above, is of significance in this connection. From this, it is clear that the etiology of pellagra and war edema can not be disposed of merely with the words, "lack of animal protein." If this were the case, then we should read of many cases of pellagra in the reports from Central Europe, which is, however, not the case. Aside from this, it is

doubtful that two such different diseases as pellagra and war edema can arise from the same cause—lack of protein. At least, in one case, there must logically be an error. *Although we believe firmly in the dietetic cause of both diseases, we cannot accept, for the present the theory that they arise from a lack of animal protein.* If this were so, then we ought to encounter much more pellagra among the millions of people that live on an exclusive vegetable diet. In both diseases, the relationship between the proteins, carbohydrates and vitamines (perhaps the caloric value of the food is often too low) seems to be incorrect, and this is all that we know at present.

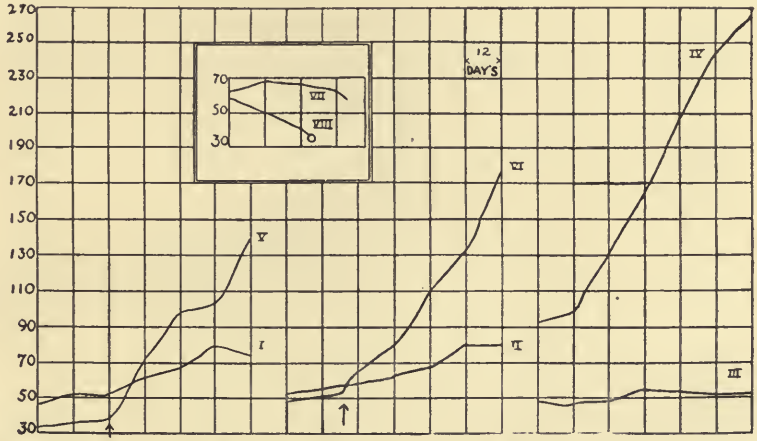

FIG. 64. THE VITAMINE REQUIREMENTS OF THE RAT ON DIETS RICH IN PROTEIN, CARBOHYDRATE AND FAT, RESPECTIVELY

I, starch; V, starch + vitamine B after 14 days; II, sugar; VI, sugar + vitamine B after 20 days; III, fat; IV, meat; VII, meat without vitamine B; VIII, starch without vitamine B; 0 = died; → = extra vitamine B given (Funk-Dubin)

We come now to the consideration of the nutrition in Europe during the war, and the effect of this diet on the general health of the people. The mode of nutrition in Germany at this time has been described by Haupt (1245), Mason (1246), Bornstein (1247) and many others. Zuntz and Löwy (1248) stated that even at the beginning of the war, robust men lost about 12 per cent of their body weight, and Rosenthal (1249) found, usually, a disturbance of the lipoid metabolism. The influence on the children and the new-born was studied by Hoffmann

(1250), Jahreiss (1251) and Bloch (1252) and Kaupe (1252a). The poor nutrition evidently had no easily recognizable influence on the intra-uterine life. The size and the weight of the new-born was not far different from the normal. As regards the children of school age, there are the reports of Abderhalden (1253), Pfaundler (1254) and Blanton (1255), all of whom speak of a poor development of the children, both in body and mind. In Belgium, the same observation was made by Demoor (1256) and Duthoit (1256a), and in France by Nobécourt (1257). Fronczak (1258) reported on the effect of the war in Poland, and Rosenfeld (1259), in Denmark and Switzerland.

An exact composition of the diet (per week) was described by Berg (1260) in 1917 in Dresden:

	grams		*grams*
Whole rye bread	1500	Pork	126
Wheat bread	350	Butter or margarine	35
Potatoes	630	Eggs	35
Kohlrabi	3500	Cheese	14
Grits	14	Fruits and vegetables	400
Sugar	21	Coffee substitute	14

This gave a daily portion of 33 grams protein (of which 4 grams were of animal origin), 10 grams fat and 1217 calories. We see from this that the food was lacking in protein, fat, calories, vitamines A and (sometimes apparently) C. Many of the observed dietary disturbances in Germany were explained by a poverty of fat, and Thoms (1261) laid great stress on this dietary component. It is quite true that a poverty of fat markedly reduces the caloric value of a diet, but this is frequently confused with a lack of vitamine A. For it is possible to live very well without fat, as has been shown again by Osborne and Mendel (1262) on rats. They demonstrated that on a fat-free mixture, in the presence of vitamine A, rats may grow splendidly and consume double the quantity of food, as compared with the usual dietary mixture. Growth in the first stage proceeds much more favorably, following a greater protein intake. Drummond (1262a) obtained the same results as Osborne and Mendel on fat-free food mixtures. These results find their replica in experiments on children and adults, which we have already described.

If the lack of fat was not responsible for the poor nutrition in Germany, how did nutritive disturbances develop? In a series of

papers, Hindhede (1263) showed how this came about. Although at the beginning of the war, Germany was in better position than Denmark, as regards its food reserves, it tried to conserve its cattle to the very last, because of the belief in the special nutritive value of animal protein; for this reason, the cattle consumed a large proportion of the food which could have been utilized by the population. This was true particularly of pigs. Kuczynski and Zuntz (1264) laid stress on this point at the time. In Denmark, on the contrary, cattle conservation was prevented by law, and as a result the Danish people had a dietary composed as follows, according to Hindhede (1265):

	grams per week		*grams per week*
Whole rye bread	1860–2036	Whole milk	700–2100
Wheat bread	420– 700	Skim milk	700–1400
Potatoes	2500–3500	Beef	175– 500
Grits	210– 350	Pork	175– 314
Sugar	448– 467	Butter, margarine	250
Cheese	125– 140	Fruits, vegetables	700
Beer	700	Coffee	35– 60

This gave a daily ration of 57 to 68 grams protein (of which 17 to 33 grams were of animal origin), 48 to 59 grams fat and 2300 to 2400 calories. This diet differed from the normal in that it contained less protein, and in the substitution of vegetable for animal protein. What was the effect of this mode of nutrition on the health of the population? The mortality dropped to 10.4 per 1000, the lowest figure of all countries. There was a definite decrease of infections, including tuberculosis, and a notable drop in cancer mortality, which fell to 50 per cent of the previous figure. Unfortunately, it is not certain how much of this improvement was due to the general dietary, and how much to the abstinence from alcohol, tobacco and coffee. Of the avitaminoses in Denmark, we have reported only on ophthalmia, which we shall speak of later on. In Germany, on the contrary, although there was a lack of vitamine A, ophthalmia was rarely noted.

Besides this, we have observations on the effect of the war diet on the general health and the various resultant pathological occurrences, made by Neuhaus (1266). In women, considerable waramenorrhea was observed by Nilsson (1267). Puerperal eclampsia was markedly decreased according to Gessner (1268). Momm (1269) and Klotz (1270) found that lactation was unfavorably

affected. Zernik (1271) noted a decrease in the resistance to drugs. The number of tuberculosis cases was considerably increased in Germany (Kieffer, 1272) and in England (1273). The contrary, however, was true of cancer (Rumpel, 1274) and also of diabetes, according to Rumpel, and to Elias and Singer (1275). The effect on eye diseases was studied by Seefelder (1276).

It is evident from what has been said, that in spite of the small amount of animal protein in the war diet of Central Europe, no outbreak of pellagra occurred; this was also commented on by S. Harris (1277). In addition, despite the lack of vitamine A, very little ophthalmia prevailed. On the other hand, there was considerable scurvy and hunger edema. We see from the well-controlled diet of the Danish population during the war, that 68 grams protein (containing 27 gm. animal protein) per day, 50 grams fat and 2500 calories completely sufficed to cover the requirements. Hindhede's experiments show that the amount of protein might even be decreased further, and could be substituted by good vegetable protein.

CHAPTER VI

Pellagra

Pellagra is a non-contagious, endemic disease of corn-eating populations, prevalent in northern Italy, Rumania, southern Tyrol and North America. The disease consists in a specific erythema of the skin, stomatitis, gastro-enteritis and profound degenerative changes of the central nervous system. The progress is acute or chronic, and the mortality is appreciable. Pellagra has been described by Bouchard (1278), Roussel (1279), Marie (1280), Roberts (1281), Schilling (1282), Niles (1283) and Harris (1284).

GEOGRAPHICAL DISTRIBUTION OF PELLAGRA

The greatest prevalence of this disease is in northern Italy, where it has been known since 1700, affecting Piemont, Lombardy, Venice and Emilia. Central Italy is much less affected while the south is almost free of the disease. It is unknown in Corsica, Sardinia and Sicily. Pellagra in Italy is evidently decreasing; in 1898, there were 3987 fatal cases, while in 1905, only 2359. The census shown as follows:

Year	*Cases of Pellagra*
1879	97,855
1881	104,067
1899	72,603
1905	55,029
1910	33,869

In Austria, especially in southern Tyrol, numerous cases were recognized between 1875 and 1905; since this time, however, they have diminshed so rapidly that the number of cases in Rovereto, according to Weiss (1285), dropped from 8053 in 1904 to 3503 in 1912, upon the introduction of potatoes. In Roumania, the number of pellagra patients were calculated at about 75,000 a few years ago; the disease seems to have increased in Serbia and Bulgaria. In England, some cases have been described by Sambon and Chalmers (1286), Box (1287) and Low and Yellowlees (1288). Still, the diagnosis of sporadic cases seems to be somewhat uncertain, though

not impossible. In France, the disease is supposed to have been recognized only since the time of Napoleon I, especially in the swampy province of Landes, but it has been entirely eradicated by Roussel (l.c. 1279).

In Egypt, especially in Lower Egypt, we find an insignificant number of pellagra cases. In Canada, cases were observed by Pinault (1289) and Rolph (1290). In North America, till a few years ago, pellagra had been steadily increasing, apparently since 1880; some investigators believe, on the contrary, that it appeared only in 1900. The progress of the disease here is much more acute than in Italy, and the mortality markedly greater. The endemia is most severe in the southern states (especially Texas, Arkansas, Louisiana, Mississippi, Kentucky, Tennessee, Alabama, Virginia, North and South Carolina, Georgia) and in Mexico. Lavinder (1291), between 1907 and 1921, counted 30,000 pellagra cases, with 40 per cent mortality. Petersen (1292) reported recently that in the United States, during 1915, there were 10,663 fatal cases; in 1916, there were 6289. During this time, in the southern states, the pellagra cases were supposed to represent about 0.5 per cent of the population, which would make about 165,000 cases. In the Canal Zone, cases were reported by Decks (1293), arising on a carbohydrate-rich diet. Tuttle (1294) has described cases in South America. McDonald (1295) observed pellagra in Antigua; Nicholls (1296), on the Island of St. Lucia; de Kock and Bonne (1297), in Surinam.

PROGRESS OF PELLAGRA

Pellagra spreads in rural districts, while the cities remain free from the disease. In Italy and Egypt, the disease attacked the poorer population; in North America, however, even well-to-do farmers who follow the usual mode of country life are not immune. According to Roberts (1298), the picture of pellagra has changed in the United States in recent years; here, the acute form resembles the Italian type, and some forms are so mild that they may be easily overlooked. The disease occurs more in women than in men; in fact, the ratio between both sexes is given variously as 2 : 1 or 3 : 1. According to Siler and Garrison (1299) and Grimm (1300), pellagra often appears after a birth and in 83 per cent of all cases, it breaks out in families in straitened circumstances.

The first attacks and relapses appear in Italy mostly early in the year—March or April; in North America, beginning in February and lasting throughout the summer into late autumn. Wellman and Sparkes (1301) have reported some winter cases.

Pellagra is no respector of age, but it is seldom seen in infants. Niles (l.c. 1283), for example, says that he has personally never seen a case under 5 years and very few under 10 years of age. According to Snyder (1302), the disease is often seen in older children; thus, 10 per cent of all cases appear before the age of 15. In one case of a diseased mother who nursed her child for 6 weeks, pellagra symptoms appeared in the child a few days later on an artificial diet. Weston (1303) found in Columbia 15 cases among children, one of which had a sick mother; the child developed pellagra shortly after being weaned. Voegtlin and Harries (1304) reported the very interesting case of a 5 months old breast-fed baby, whose mother showed no symptoms. Byfield (1305) described a pellagra-like disease in 17 children under 4 years of age, for which, however, he assumed an infectious and not an alimentary cause. According to Murphy (1306), the disease is much milder in children and is without nervous symptoms.

Pellagra is not hereditary, but frequently the children of pellagrans show stunted growth, mental weakness and other signs of degeneration. It is not contagious; never was there an infection in an orphan asylum or among the guests of a health resort; according to Goldberger (1307), there was never any pellagra among physicans and nurses of a pellagra hospital.

The course of the disease varies; there are severe acute cases terminating fatally in a few weeks, and chronic types, with recoveries and relapses, of many years duration. In an examination of 100 cases, Wood (1308) noted the first symptoms, divided as follows: skin, 100 per cent; gastro-intestinal disturbance, 77 per cent; mouth symptoms appearing sometimes before the skin symptoms. Of the latter, 97 per cent appeared on the hands and the forearms, and in 39 cases, on the hands only. The uncovered parts of the body are involved, for the most part, but in one case, the anus, and in two cases, the back was affected. According to Roberts (l.c. 1281), there are altogether four different types of the disease.

1. Acute, malignant form

Duration is from two weeks to three months. It may appear as the first attack or during the progress of the disease, or end in chronic cases with prostration, convulsions, fever and diarrhea. Continuous fever (38 to 40.5°C.); pulse 120 to 130, small and arrythmic; tongue, deep red, fissured and painful; painful stomatitis and pharyngitis; nausea; typical dermatitis; skin petechiae. In addition there are frequently tremors, convulsions, tetanus, delirium and incontinence. This type, is often called "typhoid pellagra."

2. Light sub-chronic form

Prevalent in young individuals; light cases with dermatosis and dyspepsia, disappearing when cured. Duration, 1 to 2 years; no fever. This form is very common in Italy and Roumania; less so in America. The symptoms consist of a light dermatosis on the backs of the hands and wrists, reddening of the mouth mucosa, constipation or slight diarrhea.

3. Severe, cachectic sub-chronic form

Temperature at the beginning, 37.7°C; pulse, 100. Very severe symptoms along the digestive tract; the tongue, deep red, eroded; mouth and pharynx mucosa, deep red and painful; gums, swollen and bleeding; nausea, vomiting, gastralgia, persistent serous diarrhea, loss of weight. Later, there are cerebral symptoms, mental weakness and skeleton-like emaciation.

4. Chronic form

Duration, 1 to 20 years. In most cases, three periods are differentiated. A) First period, with dermatosis and dyspepsia, as in light sub-chronic cases. B) Second period, symptoms along the digestive tract become more severe; gastralgia, tabes-like stomach crises, vomiting, diarrhea and tenesmus appears. Pulse, 80 to 100, with dyspepsia and light dropsy. Typical dermatosis; on recurrence the skin of the feet is brownish red and scaly; backs of the hands are brownish red, wrinkled and aged. To this picture there are added brain and spinal cord symptoms; uncertain walk, vertigo, tremor, epileptic and tetanic attacks, contractures, accentuated

reflexes. C) Sooner or later in the third period, there is cachexia and mental weakness, with hemiplegia or paraplegia, and finally with serous diarrhea, petechiae, muscle atrophy and incontinence. Normal temperature prevails, as a rule; only exceptionally is terminal fever observed in the last days of the disease. In addition to these four types, there are light abortive forms, which we shall describe in the consideration of expermental pellagra in man.

SYMPTOMATOLOGY AND PATHOLOGY OF PELLAGRA

1. Gastro-intestinal tract

The pellagrous tongue is of diagnostic importance: in the early stage, coated; later, deep red, fissured in the middle and around the edge, often painful. The gums, spongy, red, easily bleeding as in scurvy; sometimes there is alveolar pyorrhea. Swelling and reddening of the mouth and pharynx muscosa with aphthae or small vesiculae, pyrosis, gastralgia, nausea and vomiting occur. In an investigation of the stomach of 20 cases, Johnson (1309) found a lack of hydrochloric acid in 16. This finding was confirmed by Givens (1310), who noted, however, cases in which hydrochloric acid and pepsin were present, especially in children. Pellagrous diarrhea is of importance; in a spring attack it may happen that there will be from 10 to 20 evacuations daily, often slimy and bloody. In the final stages, the evacuations occur still more frequently, serous and colorless; indicanuria is often noted. All the symptoms along the gastro-intestinal tract appear to be of central nervous origin. Siler (1311) preferred not to regard as pellagra those cases without gastro-intestinal disturbances, though it seems to us that such a differentiation would be only artificial.

In acute cases, in the stomach, swelling and reddening with erosions, especially at the pylorus were found. In chronic cases, the mucous membrane of the stomach was pale, covered with mucous, and the muscles were atrophic. In the intestine, in acute cases, there was enteritis with ulcerations in the small and large intestine, less frequently in the duodenum; in chronic cases, according to Lynch (1312), the mucosa was pale, covered with mucus, and atrophic; the musculosa was thinned. The liver was tough, atrophic, and occasionally showed fatty degeneration.

FIG. 65. TYPICAL PELLAGRA ERYTHEMA ON THE BACK OF THE NECK (ROBERTS)

FIG. 66. PELLAGRA IN A NEGRESS (ROBERTS)

FIG. 67. PELLAGRA (ZELLER)

2. Skin

The pellagrous dermatitis is one of the most important of the early symptoms. In light forms, this dermatitis is maculo-papular; in severe acute types, vesicular with intensive reddening, edema and pain. The dermatitis appears in a typical manner symmetrically on the backs of the hands, and wrists (the pellagrous glove), then on the neck, less often on the face, and on the feet (the pellagrous shoe). This dermatitis was likened by Gurd (1313) to X-ray dermatitis. The pellagrous glove and shoe extends frequently one-third of the way up the forearm and leg respectively. On the face, the dermatitis appears on the nostrils, temples and behind the ears; sometimes, ecchymoses appear on the eye-lids. On the elbows and the forearm the skin remains rough and scaly for a long time; this protracted scaling may appear on the face, shoulders and, in fact, on the whole body. In chronic cases, the nails sometimes become grayish white, thickened and brittle. On recovery, the skin appears brownish red and dark brown on the affected places; in rare cases, this extends to the entire body.

It is of etiologic importance that in the production of human experimental pellagra by Goldberger (1314), the first symptoms appeared on the scrotum. This condition has inclined some investigators to doubt the validity of Goldberger's findings, pointing out that it had nothing to do with real pellagra. Because of this, it is important to state here that Deiaco (1315), Merk (1316), Deeks (l.c. 1293), Crosby (1317) and Wood (l.c. 1308) observed this localization of the dermatitis as the first sign in their cases.

This specific dermatitis appears to be of trophoneurotic central origin, but the influence of the sun rays cannot be altogether denied. The burning of the hands and feet, a very disagreeable occurrence independent of the dermatitis, belongs to the nervous symptoms.

3. The nervous system

Pain in the back is one of the early symptoms of severe cases. The reflexes are accentuated. The muscles become atrophic in half of the chronic cases, with fatty degeneration; contractions in a flexed position sometimes develop in the hands and feet. The degeneration reaction is usually lacking. Among the later symptoms, are tremor, especially of the hands, infrequently of the tongue and lips, and

cramps of some muscle groups; in severe cases, there is spastic or atactic gait. As terminal symptoms, there are tetanic, epileptic attacks, and paralysis of the sphincters. Mental disorders belong to the typical picture of severe pellagra. The initial symptoms are insomnia, quietness, sadness, later hypochondria and finally psychoses

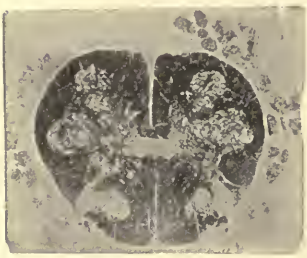

Fig. 68. Spinal Cord; Lumbar Region (Mott)

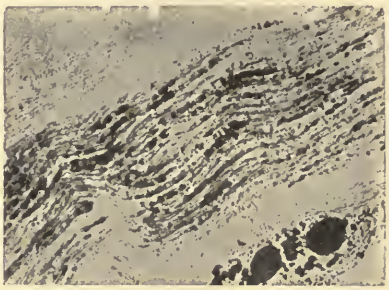

Fig. 70. Sciatic Nerve in Pellagra; Longitudinal Section (Mott)

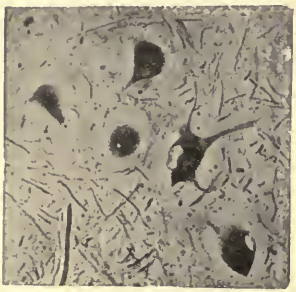

Fig. 69. Cells of Ventral Horn with Asymmetric Nuclei (Mott)

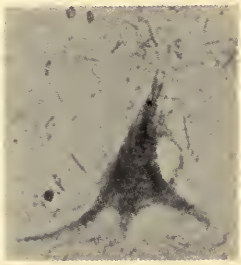

Fig. 71. Cell of Betz in Motor Cortex (Mott)

of all kinds. The latter develop in 10 per cent of all cases in Italy, in about 5 per cent in America, apparently more often in the chronic form. According to Sandy (1319) the number of pellagra psychoses among the inmates of insane asylums is very large in the southern states. Miller and Ismail (1320) investigated the various types of pellagrous lunatics in Egypt, classifying 750 cases as follows:

Amentia (42 per cent—typical first stage)	320
Mania	115
Melancholia	113
Dementia	103

The mortality was 32 per cent; aside from this, 47 per cent were discharged, with unknown issue.

In the brain, the following lesions were found: pia and arachnoid were thickened, with a milky cloudiness and ecchymoses. In the brain, mostly edema and hyperemia with hydrops of the ventricles are noted. In chronic cases, the brain and the brain convolutions, especially the frontal, frequently become atrophic, hard and anemic. Miscrocopically, cortical nerve cells are degenerated, with swelling, vacuole formation, displacement of the swollen nucleus to the side, and later atrophy of the degenerated cells.

In the spinal cord, in acute cases, dilatation of the blood vessels and edema are found; in chronic cases, chiefly degeneration of the dorsal fibers and the direct pyramidal tract. Scattered localities with disappearance of the nerve fibers in the whole white substance of the spinal cord were also noted. With this, the dorsal roots are also degenerated with arterial thickening. In the gray substance, and also in the cells of the ventral and dorsal horns, we find pigmentation, swelling of the cell protoplasm, chromatolysis and displacement of the nucles to one side. In the sympathetic nerve, especially in the abdominal ganglia, degeneration was observed. It must be explicitly stated that in the nerve centers, there was not a trace of inflammatory lesions; all the changes, according to Mott (l.c. 890), are exclusively of a degenerative nature.

Microscopic hemorrhages were often noted in the nerve centers. In one of Chalmer's (1321) cases in Roumania, the cause of death was a marked hemorrhage in the lower cervical and dorsal part of the spinal cord. In the sciatic nerve, Mott found scattered degeneration of nerve fibers.

4. Circulatory system

In the blood, according to Findlay (1318), an increase in lymphocytes is mostly found. Hemoglobin, about 80 per cent; pulse, usually 100 and in acute pellagra, up to 120; blood-pressure is mostly low. Examination of the blood for microörganisms was negative. Bardin (1322) found an increase in the number of small and large lympho-

cytes and a decrease in polymorphonuclear neutrophiles. Microscopic findings were recorded by Nagamatsu (1323). Examination of the cerebrospinal fluid, according to Lorenz (1324), gave no reason for regarding pellagra as an infection of the central nervous system.

The heart is mostly atrophic, pigmented, fragile and occasionally shows fatty degeneration. Sometimes there is lung edema, hyperemia and hydrothorax.

Fig. 72. Rarification of the Phalyngeal Ends in Pellagra (Roberts)

5. Bones

Lombroso (1325) and Babes and Sion (1326) observed, in some cases, brittleness of the ribs and long bones. Fractures of the long bones were also noted. Similar lesions are characteristic of human and experimental scurvy. Roberts (l.c. 1281) gives some X-ray pictures of pellagrous bones, showing a rarification of the bone ends.

6. Sexual organs

In acute cases, vulvo-vaginitis is not infrequently observed, and in rare cases, there is gangrene of the labia. In chronic cases, amenorrhea frequently occurs. Abortion was noted in 20 per cent of

pellagrous pregnant women; hemorrhages following birth are not uncommon. In latent cases, following pregnancy, an acute condition is not infrequently brought on; similar observations have been made in beriberi.

7. Other organs

Regarding eye symptoms, Marie (l.c. 1280) described conjunctivitis with pterygium and hemeralopia, and sometimes pigmentary retinitis. Calhoun (1327) found that certain visual disturbances, especially certain forms of color blindness, precede other symptoms of pellagra, so that they may be used in the diagnosis.

As regards the endocrine glands, Beeson (1328) observed, out of 316 cases, 25 cases of thyroid complications. Modinos (1329) noted cases in Egypt with enlarged adrenals, about twice the usual size. In cases observed by Wilson (1330), also in Egypt, these glands were much lighter in weight. Morse (1331) found severe atrophic degenerative changes in the thyroid and the adrenal medulla. In the latest reports, especially from Egypt, special attention was paid to the insufficiency of the adrenals (Wilson, l.c. 1330).

CHEMICAL PATHOLOGY

Koch and Voegtlin (1332) analyzed the spinal cord and brain of pellagrins and found that the figures agreed, in general, with those obtained in pigeon beriberi. One of the most important findings was the loss of lipoids, particularly in the spinal cord.

Indicanuria was found by Ridlon (1333) in 90 per cent of his cases. Murlin (1334) found high amino nitrogen and hippuric acid values (two to three times greater than normal), indicating a poorer nitrogen utilization. If the carbohydrate diet is replaced by a protein diet, then the values found are smaller. The effect of both of these diets on the composition of the blood was investigated by Lewis (1335). The carbohydrate diet gave smaller urea and non-protein nitrogen figures, but otherwise the findings were normal. Jobling and Maxwell (1336), as well as Sullivan and Stanton (1337), determined the alkali reserve of the blood; the figures varied only slightly from the normal; no acidosis was noted. Sullivan and Jones (1338) found indican in the saliva in one case; in addition, there was a thiocyanide reaction, indicating a retardation of protein metabolism.

METABOLISM

The first exact metabolism experiments in pellagra were made by Myers and Fine (1339). The milk-vegetable diet used was well utilized. Nicolaidi (1340) observed large food losses in the stool. Albertoni and Tullio (1341) obtained a negative nitrogen balance on corn; the balance became positive on meat. Hunter, Givens and Lewis (1342) found in seven cases of pellagra a positive nitrogen balance on a pellagra-producing diet. Boyd (1343) found, in a metabolism experiment on Turkish prisoners in Egypt, in confirmation of earlier findings, a decrease of hydrochloric acid in the stomach, diminished pancreatic secretion, protein and fat loss in the stool (remarkable in a disease caused by a lack of protein) and a very poor protein assimilation. The latter is especially emphasized in recent publications (Sullivan, Stanton and Dawson, 1343a).

PROGNOSIS

In Italy, 55,029 cases of pellagra were recorded in 1905, with 2359 deaths, equivalent to more than 4 per cent mortality. In America, on the contrary, the prognosis is much more serious, namely, 50 per cent death rate in the asylums and 20 to 25 per cent in private practice. It is obvious that the picture of pellagra, because of the dietetic factor, frequently varies with the well-being of the population, assuming either a mild or more severe type.

THE RELATIONSHIP OF PELLAGRA TO THE ACCEPTED AVITAMINOSES—BERIBERI AND SCURVY

In the short paragraph on the relation of beriberi to scurvy in South Africa (p. 296), we have seen that scurvy develops on a diet consisting of very little animal protein and corn, without a sign of pellagra. This is true also of a widespread occurrence of scurvy, which G. R. Hopkins (l.c. 939) described in a population which fed itself almost exclusively on corn. Marie (l.c. 1280) and Viswallingam (1344) looked upon scurvy as a predisposing cause of pellagra.

As for the relation of pellagra to beriberi, Sheppard (1345) saw pellagra in Singapore on an exclusive rice diet. Stannus (1346) stated that he saw a number of cases in Nyasaland on rice (only partially husked); vegetables, fish or meat were eaten only once in 14 days. The disease progressed without any characteristic exan-

thema, although certain signs of the skin affection were visible around the mouth. On the other hand, a pellagra-like erythema was observed in beriberi by Schüffner and Kuenen (1347). Nightingale (1348) saw 1210 pellagra-like cases, in South Rhodesia, which he called "zeism." The disease developed on milled corn, was mild and without exanthema, but with light dermatitis and mouth symtoms. He stated that cod liver oil therapy was quite favorable. Edwards (1349) designated as "peripheral neuritis" a disease met with in Jamaica among the poor inhabitants who live on a nitrogen-poor diet. The symptoms resemble pellagra more than beriberi. Finally, we wish to comment upon the composition of the diet, which Braddon (l.c. 866) regarded as productive of beriberi. It was exceptionally poor in animal protein and must have lead to pellagra, according to the newest conceptions. This short chapter was introduced here, not so much to demonstrate the relationship between pellagra and the avitaminoses, but to show that we may be dealing with mixed forms under certain conditions, which indicates perhaps that if the diet is lacking in one constituent, it is likely that others may be lacking at the same time.

MODE OF DEVELOPMENT OF PELLAGRA

Although theoretically pellagra may also develop without any corn consumption, it appears and disappears, in practice, with corn cultivation. Thus, corn was introduced into Egypt in 1840, and in 1847, the first cases of pellagra were noted. In Spain, the disease has been endemic for two centuries, having appeared there with the beginning of corn cultivation; at present, considerable rye, wheat and oats, but very little corn, are grown and the disease has almost been stamped out there. Weiss (1350) noted that since 1905, after the exclusion of corn from the diet, cases in the Tyrol appeared more rarely. Alpago-Novello (1351), in an Italian province, partially substituted corn growing by turnips and potatoes, whereupon the number of cases decreased. It is likewise not without significance whether hand ground or machine milled corn is used. In the United States, it has often been found that in the mountainous districts where only the hand milled variety is used no cases of pellagra occur; the contrary is true in small industrial cities where the natives use prepared cornmeal, as brought out by Wood (1352). Blosser (1353) observed

130 cases develop on a diet containing a considerable amount of cane sugar products, especially molasses. By omitting the latter, which is greatly relished in the southern states, an improvement was noted. That a diet very rich in carbohydrates is consumed in districts where pellagra prevails, is evident from the report of Jobling and Petersen (1354). Babes (1355) remarked, advisedly, that when in February 1918, the entire population of Bucharest was placed upon bread made of corn and wheat, numerous cases of pellagra appeared as early as in May, so that three months were necessary for the development of the disease. Lombroso (l.c. 1325) and Camurri (1356) describe a dietary composition in the Italian pellagra districts:

Lombroso		*Camurri*	
	Grams daily		Grams daily
Corn	1091	Polenta	1500
Rice and barley	67	Rice	100
Beans	60	Beans	100
Potatoes	67	Potatoes	100
Vegetables	250	Vegetables	100
Lard	21	Lard	20
Olive oil	33	Olive oil	10
Fish	67	Milk	100
Poultry	27	Cheese	50

Apart from this, great differences were noted in the summer and winter diets, which explains very well the periodic occurrence of pellagra in spring. It seems that the Italian population feeds itself very poorly in the winter. Lombroso reported on such dietary changes in the province of Ferrara:

	DIET IN 8 WINTER MONTHS	DIET IN 4 SUMMER MONTHS
	grams per day	*grams per day*
Polenta	1000	160
Milk		
Eggs	Almost none	Almost none
Onions	One daily	Two daily
Corn bread	50	400
Home-made bread	50	200
Meat	10	60
Cheese	5	20
Beans	150	40
Fish	20	Very little

These figures were corroborated by Devoto (1357). Wilson (1358) saw the development of pellagra on a diet containing 92 grams protein and 2200 calories. The protein consisted of ¾ wheat and ¼ corn; 10 per cent of the war prisoners developed pellagra in one year, the disease appearing only after hard labor. Boyd and Lelean (1359) observed pellagra among 6000 Turkish prisoners, to the extent of 18 per cent, while among German prisoners, there were no cases; it was only when they were assigned hard labor to perform that 65 cases appeared, according to Lelean (1360). Bouchard (l.c. 1278) noted that shepherds developed pellagra, while cow attendants remained free from the disease. The difference was supposed to lie in the milk consumed.

The mode of occurrence of pellagra was studied very accurately by Goldberger and his co-workers. Goldberger, Wheeler and Sydenstricker (1361) compared the diets of diseased and healthy natives in a small industrial city in South Carolina. The chief difference was in the milk, meat, vegetable and fruit consumption. The amount of protein was about 85 grams, of which one-third was of animal origin. The reason why other investigators did not observe the above differences is because the pre-pellagrous diet, and not that which is consumed at the time of the attack, must be studied; for in this case, the disease is already improving. Sydenstricker (1362) could show by statistical data, that the cases become more numerous, when the people, because of increased prices, must limit their expenditures. Apparently, the decrease in the number of pellagra cases in the United States during the war is due to the great prosperity of the population. Goldberger and Wheeler (l.c. 109) produced experimental pellagra in 11 prisoners. The diet, accurately controlled, was analyzed by Sullivan and Jones (1363), and the vitamine content was tested on animals by Sullivan (1364). The diet consisted of 41 to 54 grams protein, of which 80 to 97 per cent was of vegetable origin, and it seemed to be poor in vitamines B and A. The controls received a better diet. The experiment lasted 6½ months, during which time, out of 11 experimental subjects, 5 developed symptoms, which must be regarded as somewhat abnormal pellagra symptoms. The first symptoms appeared on the scrotum, which occurs sometimes, as we pointed out previously. It is apparent, according to Goldberger, that the 11 subjects had pellagra in a mild form, and that the symptoms may vary with the diet. Goldberger (1365) held that three months sufficed to develop symptoms of pellagra on a suitably chosen diet.

THERAPY

Roussel (l.c. 1279) stated in 1866 that dietetic measures were of greatest importance in combating pellagra, a view in which a great number of investigators are agreed, including Royer (1366) who expressed himself similarly in 1835. Lombroso (l.c. 1325) saw that a meat addition produces an extraordinarily favorable effect. Kleiminger (1367) cured 12 pellagrous lunatics by means of diet, and Lorenz (1368) made considerable use of the dieto-therapy. Elebash (1369) used green vegetables and fruit juices; Allison (1370) used milk, eggs and fruits; Sylvester (1371) at first used fruit juices, then skim milk and eggs. Bravetta (1372), Willets (1373) and Ridlon (1374) proceeded similarly. The latter treated 51 cases with a specially selected diet, 48 of which recovered. Goldberger, Waring and Willets (1375) tested, in some asylums, a diet consisting of milk, meat and vegetables, with a decrease of carbohydrates, and obtained very good prophylactic and therapeutic results. Voegtlin, Neill and Hunter (1376) used a vitamine therapy in their practice, with the following results; with vitamine B, no results were evident, while with vitamine A, in the form of liver and thymus extracts, some result was obtained but it was not very marked. Sherman (1377) recommended milk as a prophylactic.

As we have noted, the best results were obtained by dietary changes; in fact, the complex products designated as animal protein proved effective. We have already pointed out that with the addition of meat, eggs or milk, not animal protein alone is added, but also food complexes which are, for the most part, unknown. By this means, a diet rich in carbohydrates and poor in protein is changed to one poor in carbohydrates and rich in protein, whereupon the entire nutrition undergoes a radical change. The modern conception of the etiology of pellagra is based upon the above findings.

ETIOLOGY OF PELLAGRA

Pellagra occurs chiefly on corn. In this connection, a series of hypotheses were proposed to explain the nature of pellagra, which, however, we can not discuss here. Even the proponents of the infection theory, for example, Siler, Garrison and MacNeal (1378) partially admit that the diet also plays a rôle in this disease. Tanner and Echols (1378) point out that it is not enough merely to give a

satisfactory diet. It must also be noted that the diet is actually consumed.

As regards the nutritive value of corn, we have already discussed this matter in detail (p. 250). With the assumption that even the whole kernel possesses a low biological value, we are not in accord, since most birds can live on it indefinitely. What happens to the nutritive value of the protein, after certain parts of the grain are removed by milling, we do not know exactly. The reports from Egypt by Wilson (l.c. 1330), Boyd (1379) and Roaf (1380) have shown that in pellagra, the protein assimilation is disturbed, with simultaneous defects of the sympathetic nervous system. Wilson, in particular, is of the opinion that the biological value of the protein can not drop below 40 grams casein, without the danger of the occurrence of pellagra. As a matter of fact, the pellagra-producing diet there was equal to only 22 grams of casein.

Most of the observers of the Egyptian pellagra came to the conclusion that it is related to a lack of an essential aminoacid, probably tryptophane. Bigland (1381) adopted this view, while Enright (1382) saw pellagra in German prisoners who, in his opinion, had sufficient protein in the diet. Based upon Wilson's conception, Chick and Hume (l.c. 460) undertook to produce experimental pellagra in monkeys. These monkeys were given a diet containing all the vitamines, but varying considerably in its protein content in the form of maize gluten. Three animals were kept on this diet, whereupon an erythema on the nostrils and other symptoms developed which were regarded as pellagra, and which were improved by the administration of casein. Since in this experiment, the food and vitamine intake were not controlled, and since the study of a disease supposedly related to a lack of animal protein was, strangely enough, conducted with plant-eating animals, which normally after the suckling period never eat animal protein, we must discount the value of this experiment.

The conception that pellagra is associated with a lack of protein of high biological value, is in some respects in opposition to Hindhede's findings and the experience of specialists connected with the feeding of the populations of various countries. In Europe too, during the World War, very little pellagra prevailed despite the lack of animal protein.

Infantile pellagra, as for example in the case described by Voegtlin and Harries (l.c. 1304), might be of help in clearing up the etiology. It is indeed true, according to Eckles, Palmer and Swett (1383), that the protein content of the milk can be influenced by nutrition to a certain extent; still, the possible variations could hardly explain the occurrence of infantile pellagra, of which some instances have been observed.

We are inclined to the view of Goldberger (l.c. 109), who considered the following etiological factors as possible:

1. Partial lack of vitamines.
2. Lack of animal protein.
3. Lack of a still unknown vitamine.
4. The combined influence of all these factors.

We believe that the dilution of protein with carbohydrates increases the vitamine requirements, so that although the vitamines are present, they do not suffice. We have mentioned the possible significance of the important substance, associated with proteins, and it is not impossible that this factor is lost during the milling of corn. It would be rather premature to consider the chapter of pellagra as closed.

CHAPTER VII

Sprue

This disease, also designated as aphthae tropicae, Ceylon sore mouth, psilosis linguae et intestini, diarrhea alba, tropical diarrhea and diarrhée de Cochinchine, has been described in detail by Van der Sheer (1384), Thin (1385), W. C. Brown (1386), Begg (1387), Schilling (l.c. 840 and 980) and Castellani and Chalmers (l.c. 839). This non-infectious disease, investigated since 1776, is endemic in Asia, especially in the Malay Archipelago, Siam, Annam, Sumatra, Java, but also in India, Ceylon, China and Japan, also in Australia and occasionally in Europe. In the United States, cases were reported by Wood (1388), Boyd (1389) and Sturtevant (1390). These patients were never in the tropics, but Hiatt and Allan (1391) also observed cases, which came from the tropics. The symptoms of the disease consist in painful stomatitis with vesicles and erosions and persistent diarrhea, with grayish-white foamy stools. The disease is curable by a dietary therapy.

SYMPTOMATOLOGY

The edge of the tongue and the point are reddened, eroded, with small vesicles, and covered with ulcers. Similar erosions, vesicles and ulcers are found on the gums, lips, soft palate, uvula, and cheeks. The patients complain of marked pain on chewing and swallowing. According to Bahr (1392), the disease occurs much more frequently in women, and may be confused with pernicious anemia.

The abdomen is blown up, especially at the epigastrium, the patient complains of pressure, fullness and burning in the region of the stomach, particularly after meals; in addition, there is heart-burn, flatulency and vomiting (without nausea). On investigation of the stomach, hyperchlorhydria and achlorhydria are not infrequently found. In the morning hours, without bodily pain and without tenesmus, there is a copious, soft, foamy grayish evacuation. The fat content of the feces is markedly increased, and the findings, according to Halberkann (1393) are similar to those in a pancreas affection. According to T. B. Brown (1393a), there is a complete lack of

pancreatic ferments and the therapeutic administration of such is recommended. The number of red blood corpuscles is considerably decreased (1,000,000 to 3,000,000 per cubic millimeter); the hemoglobin index is 60 to 70 per cent. In some cases, tetany belongs to the clinical picture of sprue. In this connection, there are the newer reports of Bassett-Smith (1394) and Barach and Murray (1395), who investigated the parathyroids and found them normal.

In severe cases, muscle weakness, loss of weight, depression, and symptoms of cerebral irritation are observed. In the final and fatal stage, continuous fever is often noted. The skin becomes gray and scaly; the tongue appears smooth, rimous and atrophic; the ankles become edematous; the liver becomes atrophic; the diarrhea, more severe and the pulse, slow and weak.

PATHOLOGICAL ANATOMY

In the intestine, there is a primary congestion of the capillaries of the sub-mucosa; later, hemoglobin exudation and round cell infiltration; finally, atrophy of the intestinal mucosa. This constant finding of primary lesions in the sub-mucosa speaks against a local infectious cause of the disease. The œsophagus and the stomach are similarly affected, but less intensively. In severe chronic cases, atrophy of the intestinal wall, liver and pancreas is found.

THERAPY

Substances like opium, bismuth, etc., are rather harmful. Emetin was recommended by Schmitter (1396) although, according to Mühlens (1397), this is indicated only in cases complicated by dysentery. Simon (1398) used vaccine with good results, although he stated that a change in diet must be made simultaneously. Castellani (1398a) recommends sodium bicarbonate administration in conjunction with the dietetic treatment. According to Wegele (1399) the dietetic therapy is most productive of results. Among the best substances to use are, according to Low (1400), milk; according to Cantlie (1401), fruit, and according to Conran (1402), meat. These are the same substances used in the treatment of avitaminoses, but in sprue, special care must be exercised and only frequent small doses should be administered. Especially to be recommended, is milk, either raw or cooked for a short time, raw

meat juice, meat broth and meat jelly; of the fruits, especially strawberries (2 to 3 pounds daily), but also apples, oranges, grapes, gooseberries, and blackberries. Cantlie (1403) advised beginning carefully with the meat diet (meat juice, meat jelly) and giving milk every 3 or 4 days. An exclusive milk diet does not suffice to bring about a cure, so that in certain cases it must be combined with a fruit diet. Later, light mixed food is gradually and carefully given. Bovaird (1403a) suggests as therapy the replacing of fats and carbohydrates by proteins and fruits.

PATHOGENESIS

Since sprue occurs less frequently than pellagra the number of published communications on the subject is not very large. Although some investigators like Cantlie (l.c. 1401) and Michael (1404) believe in an infectious cause (a fungus, Monilia psilosis), nevertheless the following circumstances point to an avitaminosis: Ashford found (1405) that in Porto Rico only the city dwellers, who eat much bread, develop sprue, but not the farmers, who eat bananas. According to W. C. Brown (l.c. 1386), the Chinese coolies in East India develop beriberi, whereas the Europeans, on a practically similar diet, develop sprue. Stewart (1406) is of the opinion that no real basis exists for regarding pellagra and sprue as two different diseases. Heaton (1406a) believes rather in the dietetic than in the infectious origin of sprue. Werner (1407) observed scorbutic symptoms (petechiae on the calves of the legs) in sprue. In the early stage of sprue, there is often an aversion for meat and this feeling, together with the known fear of fruit in diarrhea, leads to the further development of the disease. Still, the most important reason for considering sprue here is the influence of dietary changes on the course of the disease, and the therapeutic results following the administration of vitamine-containing foodstuffs. In particular, the fact that emphasis is placed upon the raw state of the foodstuffs in the treatment gives further support to our conception. Especially in the use of strawberries, does Leede (1408) emphatically state that preserved strawberries are far less effective than fresh berries, and that the active substance contained therein is destroyed on heating. Whether we have, in sprue, a disease similar to pellagra or scurvy, cannot be stated with certainty at present; we must await further work on the subject.

CHAPTER VIII

Hunger Edema

Hunger edema had been observed, prior to the late war, and Sticker (1409) and Prinzing (1410) occupied themselves particularly with the history of this disease. Prinzing, in his historical compilation, records no cases between the time of the Peloponesian War (430 to 425 B.C.) and the siege of Port Arthur in 1904. Maliwa (1411) believed that hunger edema was observed in the Napoleonic Wars, at the siege of Paris, and in the Boer War. Wheeler (1412) described actual cases in the Boer War, in the concentration camps. Digby (1413) and McLeod (1413a) reported on this disease during famines in India and Ceylon in 1876 and 1877. Patterson (1414) saw numerous cases in Chinkiang (China) in 1899, during the time that the population was living on greens. Landa (1415) counted hundreds of cases in Mexico during the war in 1915, which developed on a diet of turnips and spinach.

In the last war, the first report on the subject was made by Rumpel (1416). Then, in rapid succession there came numerous reports of the disease among prisoners by Jürgens (1417), Bönheim (1418) in Bonn and Lange (1419) in East Prussia. From Austria, among other reports, were those of Knack (1420), Schiff (1421) and Jaksche (1422); indeed, in one part of Bohemia alone, 22,000 cases were noted, with 4 per cent mortality.

Vandervelde and Cantineau (1423) and Breuer (1424) reported on cases in Belgium. Beyerman (1425) observed cases in the insane asylum in Medemblik (Holland) in 1917 and 1918, which were cured by fresh vegetables; in these cases there was perhaps a complication with scurvy. Strauss (1426), Guillemin and Guyot (1427) and, at the beginning of the war, Budzynski and Chelchowski (1428) described the disease in Poland. Wells (1429) saw numerous cases in 1917 in Roumania; Tonin (1430) observed the disease among prisoners in Italy; Enright (1431), among Turkish prisoners in Egypt.

Mann, Helm and Brown (1432) noted 3000 cases in Haiti, out of which 200 came to necropsy. The disease has prevailed there for

years among the prisoners, in fact, it was noted that the disease broke out in less than three months after incarceration.

In infants, on a carbohydrate-rich diet, there develops a condition called "Mehlnährschaden." of which we have already spoken. Many of these cases, if not all, can be considered analogous to hunger edema. Vacher (1433) reported such cases during the siege of Paris; later, similar conditions were described by de Wolf (1434) and Potter (1435) in children suffering from marasmus. Potter believed that lack of protein was the cause of the disease. The disease was also noted by Chapin (1436), Waterman (1437), Hume (1438), Ashby (1439) and Klose (1440). Many of these investigators studied the effect of a change in the diet, especially an addition of protein. In the American literature, the disease is often designated as acrodynia and has been described by Weston (1440a) and by Cartin (1440b).

Of additional interest, is the relationship between hunger edema and "epidemic dropsy" in India. This disease, described by Greig (l.c. 880), was first regarded as wet beriberi. Although scorbutic symptoms were also observed, the condition of the patients could be markedly improved by an addition of meat. Most of these cases developed on a rice diet, and yet they could be identified as hunger edema. McCay (1441) believed that the cause of epidemic dropsy lay in the lack of protein. In a compilation on this subject, Maver (1442) believed that hunger edema, edema of children and epidemic dropsy have the same etiology.

SYMPTOMATOLOGY AND MODE OF DEVELOPMENT

A splendid description of the symptomatology, pathology and pathogenesis is given by Schittenhelm and Schlecht (1443) and by Schiff (1444). The most prominent sign is the edema, localized mostly on the legs and on the face, near the eyes. According to Hülse (1445) pains in the calves are very characteristic. Polyuria is very pronounced, according to Rumpel and Knack (1446) and Zondek (1447). Jansen (1448) found decreased blood pressure and subnormal temperatures. Jess (1449) called attention to the eye symptoms, hemeralopia and peripapillary edema of the retina. The marked bradycardia is especially characteristic of hunger edema, according to Rumpel and Knack (l.c. 1446). This is not to be at-

tributed to heart weakness, according to Schittenhelm and Schlecht (l.c. 1443), but to the same causes that bring about the edema. In these cases, adrenaline dose not raise the blood pressure, and the usual heart stimulants, such as digitalis and caffeine, have little effect, while atropine has no effect on the bradycardia. The pulse is extraordinarily small, slow (32 to 36) and soft; reflexes and normal; constipation was noted more frequently than diarrhea, though the latter progressed as the edema disappeared. The disease appeared mostly in hard-working men; the over-exertion seemed to hasten the onset of the disease. Frequently, rest in bed sufficed to dissipate the edema and to bring about an improvement. The disease is often found associated with various infections, such as lobar pneumonia, bronchitis and furunculosis.

Various investigators offer different explanations of the mode of development of the disease, though there is no doubt that the poor diet must be the primary cause; the predisposing conditions may be severe manual labor and exposure. Knack and Neumann (1450) stated that the cause was inadequate nutrition with turnips and a large water intake. Falta (1451) thought that the faulty diet was bread, cabbage, dried vegetables and turnips. The total diet contained 30 to 59 grams protein and 1200 to 1400 calories; the vegetables were cooked in large quantities for 4 to 7 hours. The cases reported by Falta occurred among war prisoners. Bürger (1452) gave the exact composition of the faulty diet; it consisted of 55.9 grams of protein, 8.5 grams fat and 284.6 grams carbohydrate (1478 calories). Kraus (1453) observed cases developed after hard labor, on a diet containing 15 per cent indigestible carbohydrates, little fat and a maximum of 50 grams protein, and 800 to 1300 calories.

PATHOLOGY

On autopsy, a complete disappearance of subcutaneous fat tissue was found, although well-nourished bodies also came to necropsy. Atrophy of all parenchymatous organs was observed; the liver and muscles were glycogen-free. Hülse (1454) found atrophy of the heart; in a few cases the adrenals were enlarged; however, according to Lippmann (1455), no pathological changes in the nerves were observed. According to Paltauf (1456) the anemia is very insignificant. In the pre-edematous stage, according to Jacobsthal (1457)

high hemoglobin and erythrocyte values were found. Woltmann (1458) found anisocytosis, polychromatophilia, high leucopenia, lymphocytosis and hydration of the blood. In epidemic dropsy, Maynard (1459) found increased intra-ocular pressure.

METABOLISM

Schittenhelm and Schlecht (l.c. 1443) conducted metabolism experiments with a diet regarded as productive of edema (45 to 55 grams protein and 1100 to 1800 calories). It was soon established that a characteristic assimilative failure was not shown by the patients, since they gained weight on more protein and fat and retained nitrogen. Zondek (l.c. 1447) had some patients who excreted six liters of urine daily, with 40 grams, and more, of sodium chloride, accompanied by a normal nitrogen content. Phosphoric acid excretion was high (4 to 5 grams); Hülse (l.c. 1454) was able to demonstrate a good protein assimilation, but believed that the de-aminization of the amino acids was faulty. Franke and Gottesmann (1460) studied the rate of elimination of urea, sodium chloride and other salts, and found a retardation. The pathological chemistry of hunger edema was considered by Feigl (1461), who studied the cases of Rumpel. Schittenhelm and Schlecht (l.c. 1443) found the protein content of the blood low, 4.5 to 5.7 per cent instead of 7 to 9 per cent. The erythrocytes showed a lowered lipoid content. In the opinion of these investigators, the low figures are not to be attributed merely to the dilution of the blood, for as the edema disappears, the blood values drop still more. The blood sugar is either normal or increased. The composition of the edematous fluid resembles that of exudates. At the height of the edema, a retention of water and chlorides is noted. On the disappearance of the edema, considerable urea and sodium chloride excretion is found, which is increased still more by thyroidin and decreased by adrenaline. Pituitary extracts are effective, but only on the sodium chloride excretion.

THERAPY

Here too, there is no uniformity in the literature. Zak (1462) believed that hunger edema could be cured not only through the addition of animal products (eggs, raw calves' liver), but also by means of carrot juice. Maase and Zondek (1463) were of the opinion that the addition of 100 grams fat could arrest the progress of the

disease. Schiff (l.c. 1444) sought the cause of the disease not in the lack of calories, per se, but of potatoes, fresh vegetables and milk; addition of yeast, beer and fat had no therapeutic effect. Knack and Neumann (l.c. 1450) obtained good results with potatoes. Reach (1464) made use of organotherapy, in the form of testicles, with good results. v. Hösslin (1465) and Schittenhelm (1466) believed mainly in the effect of protein therapy. Isenschmid (1467) attributed certain edemas, like those occurring after dysentery and other protracted diseases, to the same causes that bring about hunger edema, and in his opinion, they should be treated in a similar manner. Most investigators manifest a particular belief in protein of animal origin as a therapeutic measure. The above therapy constitutes the sum and substance of the leading ideas as regards the etiology of hunger edema.

ETIOLOGY

Because of the great lack of fat during the war, some investigators associated the etiology with this factor. We have just seen that this is not likely and that it has to do chiefly with a lack of vitamine A, and most animal products used contain more or less of this substance. Aron (1467a) considers that hunger edema is due to an insufficient amount of calories in the diet. Park (1468) was of the opinion that the edema was associated with a lack of protein and fat, and an excessive water intake. Rubner (1469) and Determann (1470) attributed it to the lack of protein. The latter conception is also in accord with the experience gained in the studies on parenchymatous nephritis by Epstein (1471) and on renal edema by Allbutt (1472). Further confirmation is given by the study of the development of experimental edema in animals, especially of rats and mice. Tachau (l.c. 1244) stated that when the correct relationship between proteins and carbohydrates is disturbed by the addition of the latter, edema develops. Denton and Kohman (1473) observed that when rats are fed on carrots, casein, starch, butter or lard and salts, this diet is adequate only when the carrots provide half of the total calories. However, if this relationship is changed by the addition of starch or fat, then edema develops in a great percentage of the animals. Kohman (1474) carried this idea further, in that by means of the above diet, especially with the addition of water, the animals developed edema, which was cured by the administration of casein. No exact figures are given as to the food intake, but in any event,

we have already voiced our opinion, in the introduction to this chapter, against the conception of a lack of protein as an etiological factor in the development of hunger edema. By the addition of casein, it is possible, first of all, that there might have been an increase in appetite, with a consequently greater food and vitamine intake. The considerable quantity of water not only diluted the vitamine and protein content of this diet, but also caused great loss by washing out. Kohman tried to get around these difficulties by showing that on a synthetic diet prepared in the usual manner, a small food intake, per se, is not followed by edema. However, since the diet was properly constituted, in this case, the results cannot be applied to the carrot diet.

Another hypothesis deals with the rôle of the adrenals in the development of edemas; this was formulated by McCarrison (l.c. 290 and 608). He showed that in beriberi, there was an increased adrenaline output which was supposed to be responsible for the development of edema. McCarrison held that butter, but not all fats, contains a substance (apparently vitamine A) which is protective against edema. This idea was adopted by Bigland (1475), in a practically unchanged form, who regarded an increased or decreased suprarenal activity as the causative factor in the explanation of hunger edema and pellagra, respectively. Nixon (1476) assumed, as the cause of edema, insufficient calories (fat?) and protein and excess of water; adrenaline was also thought to play a part. In conclusion, we shall mention the pathogenesis assumed by Schittenhelm and Schlecht (l.c. 1443). They ascribed the cause to the lack of protein and fat, the effect of cold and hard labor helping to bring about the disease. They recognized that hunger edema occurs on a diet rich in carbohydrates. The differentiation from beriberi lies in the bradycardia; on the other hand, scurvy-like symptoms, such as stomatitis and bleeding of the gums were observed. Hemeralopia and xerosis of the eyes were also noted quite frequently. Administration of vitamine B exerted no therapeutic effect. There is a great analogy to "Mehlnährschaden."

Our presumption is that we are dealing here with a complication of causes, although the disproportion between the individual dietary constituents, as well as an inadequate diet in relationship with the hard labor stands in the foreground. However, in this case too, further work is necessary in order to arrive at an explanation of the problem.

CHAPTER IX

Pathological Conditions in Which the Lack of Vitamines May be Suspected

It is difficult, if not impossible, to determine in the conditions mentioned below, at the present time, if the described effect is due to the vitamines, per se, or to the other nutritive constituents administered simultaneously. The subject may be cleared up only when we shall have the vitamines available in larger amounts in the pure or almost pure state.

KALLAK

Little (1477) reported, in 1908, on a disease called "kallak," occurring among the Eskimos in New Foundland. The disease consisted in a marked pustulous dermatitis on the hands, elbows and other parts of the body, with persistent itching. The disease is usually curable. The natives already know, from experience, that the disease makes its appearance when only a small quantity of seal meat is available. It occurs in autumn, when they live mostly on fish and when wild berries become scarce. Their diet consists of seal meat, carribou, birds, fish and berries, and is very rich in protein. The disease is very predisposing to scurvy, and Little believed that it might be an avitaminosis.

TRENCH SICKNESS

Bruntz and Spillmann (1478) observed the disease among the French soldiers in the trenches. It consists partly of neuritic symptoms (parasthesias, pains, motor disturbances, asthenia), and scorbutic symptoms in the mouth, especially the gums. Following a better diet (fresh vegetables and fruits), the number of cases decreased markedly. It is related to scurvy, with a partial lack of other vitamines, and is made more severe by cold and over-exertion. Psychic phenomena and uncleanliness apparently also play a great rôle in the pathogenesis. All the extremities were affected. This disease was also studied by Mercier (1479) and Chauvin (1480).

INTESTINAL STASIS, ETC.

Following his interesting findings in the intestines in avian beriberi, McCarrison (1481) sought to apply his results to an explanation of some obscure intestinal disorders. He thought for example, that properly nourished children showed less tendency towards intussusception. Among the diseases which McCarrison associated with the vitamines is intestinal stasis, which was treated by Sir Arbuthnot Lane (1482), and later, intestinal toxemia and the so-called "coeliac disease," described by Still (1483). The latter observed, among 41 cases, 4 that had scurvy previous to developing coeliac disease. As an indication of the lack of vitamines, McCarrison pointed out dilatation of the stomach, air pockets in the intestine, impairment of the neuro-vascular control of the gastro-intestinal tract, formation of stomach ulcer and especially colitis. Mackenzie Wallis (1484) adopted the view of McCarrison in regard to coeliac disease, in that he made use of vitamine therapy in this condition among children, with good results.

STERILITY

Reynolds and Macomber (1485) found that when rats are fed on inadequate diets (also in the case of vitamine deficiency) they exhibit a marked diminution in fertility. This viewpoint which is well borne out by laboratory experience has been considered in the treatment of analogous conditions in man and favorable results have been reported.

HEMERALOPIA

We have already seen that in the various human avitaminoses, hemeralopia was frequently observed. This is not associated with any particular vitamine, but arises unspecifically, perhaps on any kind of inadequate diet. We have nevertheless found data in the literature pointing to a widespread occurrence of this eye affection, which was favorably influenced by cod liver oil. Ishiwara (1486) saw such cases in Japan. He studied the lipoid content of the blood and found it decreased. When the disease was cured, the figures returned to normal. Tricoire (1487) observed among French soldiers, 320 such cases which were promptly cured by cod liver oil. H. Smith (1487a) is of the opinion that hemeralopia has mostly been simulated during the war since the visual field was not diminished as

in true cases. McCollum, Simmonds and Parsons (l.c. 1222a) regard hemeralopia as of dietetic origin, and curable with cod liver oil. Appleton (1487b) considers hemeralopia and snow blindness, as it occurs in polar regions (Labrador), due to the strong sun rays on the basis of a nervous disturbance and supposed to occur only in men who work in the open. Pick (1488) and Feilchenfeld (1489) reported on the influence of war diets upon various eye affections.

EXOPHTHALMIC GOITRE (GRAVES DISEASE)

In the study of experimental rickets in dogs, Edward and May Mellanby (l.c. 408a and b) made the interesting observation that on feeding certain fats, like butter, a hyperplasia of the thyroid develops which can be cured by cod liver oil. This observation has been made use of in human cases and good results have been obtained, though the number of cases up to the present have been insufficient to permit of definite conclusions being made.

THE SIGNIFICANCE OF VITAMINES IN INFECTIONS

Since an inadequate diet, especially the deficiency in vitamines, lowers the resistance towards infections, an insufficient production of antibodies was first thought of as an explanation. The question has been studied experimentally by some investigators. Hektoen (1490), in rats on artificial diet, and Zilva (1491), in rats and guinea pigs on a vitamine-poor diet, observed a normal formation of antibodies. On the other hand, according to Kleinschmidt (1492) the hemolysin formation does not attain its normal value on an unsuitable diet. It is well known that the method of feeding plays an important part in various infections, as has been shown by E. Thomas (1493), Valagussa (1494) and Peiser (1495). The latter reported especially on the influence of the dietary fat on the resistance to infection. During the course of our discussion, we have already pointed out the possible relationship between the vitamines and some infections, and we must call attention to the fact that since the recognition of the nature of ophthalmia, this matter has assumed greater significance. The known data on ophthalmia indicate perhaps, that they may be partially applied to other infectious conditions. For here, we have a local infection that may be influenced specifically by a vitamine therapy.

A further field in which the vitamines may be of significance, is the diet in various acute, and especially chronic infections. During convalescence, after acute infections, the diet is of greatest importance. It happens occasionally that in all these conditions, quite a one-sided diet is given for weeks at a time, for example, flour preparations, because of which recovery is impeded, and the resistance of the patients is further decreased. These conditions occur especially after typhoid fever, as Morrison (1496) showed. The question of the diet in typhoid was studied by Barker (1497), while Coleman (1498) Walton (1499), Carter, Howe and Mason (1500) laid emphasis on the caloric value of the diet in convalescence. Coleman spoke of the necessity of a well-balanced diet, with which he treated 222 patients, with the result that the mortality was 50 per cent less. Combe (1501) described the influence of the war diet on the course and convalescence of typhoid in Germany.

Isenschmid (l.c. 1447) believed that some edemas, which occur after dysentery or other protracted diseases, are of dietary origin and belong to the type of hunger edema. In all these cases, it is important that the diet, even though small in quantity, in accord with the appetite of the patient, must be complete in its composition. It is not without reason that milk is so often used for the above purpose. If the disease or convalescence is too long drawn out simultaneously with an unsuitable diet, then it is possible for a real avitaminosis to develop.

OPHTHALMIA

The disease, likewise called "xerophthalmia," seems to occur also endemically. Thus, Hirdlika (1502) described a similar disease among the Indians in the southwestern part of the United States, while McCarrison (1503) stated that the disease occurs not infrequently in India, on a diet of rice and vegetable oils, and is curable by cod liver oil. The disease consists in an infection of the conjunctiva, which, under certain conditions, may lead to a destruction of the cornea, falling of the lens and the iris, and total blindness. Mori (1504) was one of the first to describe the condition somewhat more in detail. An outbreak of this disease, called "hikan," which developed at a time of partial famine, was mentioned by Mori, who investigated 1400 cases in detail. It rarely occurred among fishermen, and could be cured with chicken liver. Czerny and Keller

(1505) observed the development of the disease on a carbohydrate-rich diet. Falta and Noeggerath (l.c. 18) were perhaps the first to produce this disease experimentally in animals. Later, this condition was observed in rats by Knapp (1506). Already at that time, he believed that a similar condition could occur in children. Since 1913, there have appeared in rapid succession, the papers of Osborne and Mendel, as well as those of McCollum, already mentioned, the cause of the disease being associated with the absence of certain fats in the diet. In the end, this disease was regarded as an avitaminosis, caused by the lack of vitamine A. This eye affection was also studied in Germany by Freise, Goldschmidt and Frank (1507), working on rats. Goldschmidt (1508) stated that the disease could be cured by a small amount of skim milk. Later it was also produced in mice and rabbits and although Nelson and Lamb (1509) stated that they could not produce it in guinea pigs and chickens, we have information to the effect that ophthalmia has been produced in guinea pigs. We have personally seen numerous cases in chickens, terminating in total blindness. Recently, we have been corroborated by Guerrero and Conception (1510), who observed the disease in chickens fed on white rice.

In recent years, ophthalmia has been frequently noted in children. A particularly careful investigation was made by Bloch (1511) in Copenhagen. He observed, during a period of 5 years in the children's clinic, 40 cases among children fed on a highly centrifuged milk, fat being given in the form of vegetable margarine. A cure was obtained with whole milk or cod liver oil. The diet in these cases appeared to be inadequate also in other respects. Bloch (1511a) believes that ophthalmia occurs quite frequently in Denmark and may often be the cause of blindness. Monrad (1512) and Rønne (1513) reported on the same condition in Denmark. In addition, one case was reported in France by Sztark (1514) and in the United States by Parker (1515). Sztark's case was a 27 months old baby fed on vegetable soups, without milk; cod liver oil effected a cure in about 10 days.

The nature of the disease

Lately, the investigators have been more and more inclined to assume that ophthalmia is associated with a lack of vitamine A. The pathogenesis of this disease is not so very simple, since we are

dealing here with a new conception in pathology. According to Macfie (1516), it is a non-contagious infection which may be prevented and cured by diet. Funk and Macallum (l.c. 86) reported that they could prevent this eye disease by antiseptic treatment, and this was concurred in by Bulley (1517). Later, however, from the same laboratory in which Bulley worked, the report of Stephenson and Clark (1518) appeared refuting Bulley's findings. They believed that Bulley's results were due to the contamination of the casein by vitamine A. In these cases, the eyes were also examined bacteriologically. The infection was shown to be unspecific, and it is of interest that before the development of the disease, no histological changes were found to account for the slight resistance towards the infection. In addition, Stephenson and Clark found that not all animals lacking vitamine A develop ophthalmia. Wason (1518a), who called the disease "ophthalmia," arrived at the same conclusion on the basis of her findings. The specificity of ophthalmia, as being due to lack of vitamine A, has recently been studied by Emmett (1519). His statistical data are as follows:

1. Without vitamine A—122 rats; ophthalmia in 120 = 98.3 per cent.
2. Without vitamine B—103 rats; ophthalmia in 0 = 0.
3. Normal diet—216 rats; ophthalmia in 0 = 0.

This disease could not be transmitted from one animal to another. Mendel (1520) observed no ophthalmia among 7000 rats fed on insufficient protein, and in 225 rats which were given no B-vitamine. However, out of 136 rats receiving no A-vitamine, 69 developed the disease. The author has personally never worked with great numbers of rats; we have had usually about 30 rats at one time under observation, but on several occasions we have seen ophthalmia in the presence of sufficient butter or cod liver oil. Thus, Funk and Dubin (l.c. 331) have recently noted two such cases out of 30 rats. These two manifested intermittent ophthalmia, lasting for a short time, disappearing, and re-appearing in a few days, but permanent lesions were recognizable. This form was favorably influenced by yeast. It is worthy of note that Guiral (1521) reported ophthalmia in children, who were completely cured by orange juice in 8 to 10 days. We are in possession of the private data of specialists who observed the eye condition in the presence of vitamine A, which was favorably affected by orange juice. Still, it must certainly be

admitted that the absence of vitamine A favors the occurrence of ophthalmia in quite a specific manner, while contrary findings require further explanation. It is possible, for example, that in the presence of cod liver oil, but with a small food intake there might be insufficient vitamine A supplied to the animal. All substances containing vitamine A may be used in the therapy of ophthalmia.

TUBERCULOSIS

That diet plays an important part in the therapy of tuberculosis, is too well known to require further discussion. An added confirmation of this is found in the reports on the influence of the war diets, of which we have already spoken. However, we may mention once more the communication of Adams and Hamilton (l.c. 1048), dealing with the increase in tuberculosis in Germany during the war. Hamburger (1522) also, in his report on the war diet of children, pointed out the dependence of the number of cases of tuberculosis on the diet, stating that it was due to the lack of fat. Stölzner (1523) likewise reported on the effect of the diet. Geoghegan (1524) reported on some cases in the West Indies, which, in his opinion, are due to the prevailing poverty. On these islands, with good climatic conditions, there should be very little tuberculosis. Richet (1525) believed that meat juice contains ferments which act favorably on tuberculosis. Woodcock and Rustin (1526) stated that the diet in tuberculosis must be rich in protein and fat (in the form of milk or milk products). If margarine is used, it must be oleo-margarine which contains vitamine A; the diet must also contain plenty of fruits and vegetables. Gardey (1526a) finds that certain symptoms of pulmonary tuberculosis resemble those of a deficiency disease. He suggests that collecting of statistical data on the relationship between the consumption and the composition of the diet in various countries would be helpful in the study of this condition.

Just how important the diet is in tuberculosis, is shown by the experimental investigations of Weigert (1527). He showed in young pigs that on a constant protein content, tuberculosis develops more frequently in the presence of carbohydrates than in that of fats. Similar investigations were made by E. Thomas and Hornemann (1528), who showed, in pigs infected with tuberculosis, that the animals manifested tuberculosis less on protein and much more on carbohydrates, while on fat, varying results were obtained. In

some communications on tuberculosis, particular attention was called to the significance of the vitamines. Rénon (1529) said that on vitamine-poor diets, a decreased immunity towards tuberculosis is evident, and Muthu (1530) likewise believed in the important rôle of the vitamines in the development of this disease.

LEPROSY

Dutton (1531) recently stated that in the development of leprosy the diet, especially the vitamines, plays an important rôle. Sir Jonathan Hutchinson (1532) believed that leprosy was associated in some way with fish consumption. On perusing the literature, we see in an article by Deycke (1533), that there is no basis for this assumption except, perhaps, the statement that in the treatment, a satisfactory diet is of significance. Among other known facts, we see in a paper by Underhill, Honeij, Bogert and Aldrich (1534) that in leprosy, certain chemical changes in the bones are apparent, indicating, perhaps, a disturbance in the calcium and magnesium metabolism. The atrophy of the bones in leprosy is associated with the large requirements of these patients for calcium. If calcium is added, marked retention results. We may, perhaps, associate this with the action of vitamine A. Vokurka (1534a) observed an increase in the number of lepers in Bosnia and Herzogowina after the war, and attributed it to malnutrition. It is obvious that leprosy occurs largely among the poorer people, but any possible relationship with the vitamines has not been demonstrated. Nevertheless, it would be worth while to go further into the question of diet.

PNEUMONIA

The many investigators in the field of experimental vitamine research have quite regularly reported the frequent occurrence of infections of the respiratory apparatus on diets poor in vitamines. Similar observations have been made in man by Fleming, Macaulay and Clark (l.c. 910) in South Rhodesia. Great epidemics were noted there on a diet, previously described, which apparently leads to a mixed avitaminosis. Vaccine therapy was used without success, and there were 686 deaths out of 2251 cases in 1908, with considerable meningitis and tuberculosis. At the same time, the avitaminosis was evident in 100 out of 700 laborers. Here, when a change was made in the diet, splendid results were obtained. Not only did the avitaminosis disappear but the pneumonia decreased also.

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CHAPTER X

Influence of Nutrition (Vitamines) on the Action of Some Poisons and Upon Pathological Conditions of Non-Infectious Origin

The older papers of Reid Hunt have already shown that changes in the diet are not without effect on the toxicity of different drugs. Similarly Reach (1535) has shown that the resistance towards the cramps-producing poison, picrotoxin, is greater in mice on a meat diet than on a bread diet and he assumed that he probably had to deal with the action of some unknown substances. Salant (1536) and Salant and Swanson (1537) observed that young carrots had a far greater protective action against the poisonous effect of sodium tartrate in rabbits, than did old carrots. The same results were obtained with sweet potatoes and carrot leaves. On oats and sugar, the toxicity was markedly increased, and the differences found were not due to increased diuresis. Cats behave otherwise, no difference being apparent; here too, however, the effect of the poison was increased about 40 per cent in starvation.

ANEMIA, ETC.

Here we have the work of Pearce, Austin and Pepper (1538), who studied the influence of the diet in anemia, after splenectomy. They observed in dogs that raw meat acts much better than cooked, and they thought this difference was due to the vitamine content. Madsen (1539) is of the opinion that the vitamines play a part in chlorosis and anemia. Geiling and Green (1539a) subjected this question to experimental test. If rats were given a diet poor in protein vitamines or salts, then the blood regeneration after a hemorrhage was markedly retarded. Davis, Hall and Whipple (1540) produced necrosis of the liver by chloroform and studied the effect of different diets on the regeneration of liver cells. Muscle tissue and fats of various sources were without effect but feeding of liver, kidney and brain resulted favorably.

Campbell (1541) and van der Bogert (1541a) thought that the occurrence of adenoids in children is of dietetic origin, but we do not

know how correct this assumption may be. The significance of vitamines in certain orthopedic cases is based on a better foundation. Schödel and Naumwerk (l.c. 998) believed that scurvy predisposes to coxa vara and other conditions, diagnosed as congenital hip-joint dislocation. Hess (l.c. 1022) saw, in one case of scurvy, a bone affection similar to coxa vara. During the war, Hammer (1542) observed that some fractures healed with great difficulty, a disturbance in metabolism being justifiably suspected. Peckam (1543) believed that some deformities like scoliosis, bow-legs, flat feet, etc., are of dietetic origin. He thought that most orthopedists are so occupied with the purely orthopedic aspect of their cases that they pay absolutely no attention to the practical important etiological factors. The presumptions of Peckam are certainly partly justified because of the relationship between rickets and bone formation.

We shall now discuss two diseases which are related more to a luxury consumption than to an insufficient dietary, namely, diabetes and cancer.

DIABETES

We see already the justification for the conception that this disease is related to luxury consumption in the papers on the effect of the war diet. Magnus-Levy (1544) observed that during the war, there was a marked decrease in the number of diabetes cases, whereas, before the war, they had been constantly increasing. Gerhardt (1545) had the same experience in Würzburg. Two phases of the diabetes question interest us here. First, the possibility of an antidiabetic substance of a vitamine type in the food, and second, the danger of an altogether rigorous dietary restriction in the usual therapy. The first possibility was justified, to a certain extent, for a specific action on diabetes was ascribed to v. Noorden's oatmeal cure. Even Magnus-Levy (1546) tried to concentrate this hypothetical substance by alcoholic extraction, but without success. Boruttau's (1547) experiments resulted differently. He found that pancreas extracts, yeast and yeast extracts inhibit the cleavage of glycogen in the isolated heart, while extracts from the peripheral oat layers decreases the sugar elimination in diabetic dogs and in man. Similar results were obtained by Rose (1548) with one of the substances isolated from the pancreas. The composition of the diet, especially its carbohydrates,

influences the blood sugar content not only of diabetics but also of normal subjects. This was reported on by Jacobson (1549), v. Moraczewski (1550) and McCay, Banerjee, Ghostal, Dutta and Ray (1551). The latter described conditions in India which develop there on a diet rich in carbohydrates, and which gradually lead to diabetes. These conditions strongly resemble experimental glycosuria, which we have already described in pigeon beriberi.

Considering the question of therapy in diabetes, we see the possibility of producing an avitaminosis by adhering too closely to a strict diet. Bürger (l.c. 1452) and Schittenhelm and Schlecht (l.c. 1443) state that in diabetes, on an oatmeal diet, they observed edema resembling hunger edema. A similar observation was made by Wilder and Beeler (1551a). An editorial in the *Journal of the American Medical Association* (1552) warned of the dangers of a decrease in the protein of the diet with a simultaneous increase of the carbohydrates, a regime which produces real diabetics from latent cases. In addition, there is the actual danger of an avitaminosis. As regards the starvation therapy in diabetes, we have asked ourselves the question as to whether the organism, in starvation, suffers first from a lack of vitamine or of the usual dietary constituents. The author and Dubin (unpublished data) undertook to test this question experimentally. However, no difference was noticeable in the time of survival between the pigeons receiving either vitamine A or B, or both, and those that were starving. In addition, no beriberi was observed in the starving pigeons.

CANCER

Cancer is a disease of excessive nutrition, although McCarrison (1553) believed that under certain conditions, a lack of vitamines may lead to cancer of the stomach. He saw one such case among monkeys. That this disease is associated with luxury consumption, is best recognizable from the statistical figures compiled during the war and from insurance statistics. Hoffmann (1554) said that cancer occurs more frequently in cities than in the country, and more among the well-to-do than among poor people, the reverse being the case in tuberculosis.

The cancer question is of interest from two points of view, first, the possibility of a chemical substance as an etiological factor and second,

the influence of the diet on the growth of neoplasms. As for the first factor, we can not take it up in such great detail as we should like to, unfortunately, since it does not fall in the sphere of this book. We were one of the first to point out this possibility, but we must admit that aside from the influence of the diet on the growth of experimental tumors and a possible chemical cause of certain neoplasms, such as the chicken sarcoma of Rous and analogous tumors, we have no firm basis for our conception. If we (1555) choose this line of thought in preference to the other known ones, it is because cancer research, in the hands of pathologists, has yielded very little tangible results. Some of the known pathologists, like Ewing (1556) and Leo Loeb (1557) were inclined to a similar view at one time. Certain experimental papers like those of Calkins (1558), who reported on the stimulation of protozoa (Didinium nasutum) by tumor extracts, may be interpreted in the above manner; still, it is possible that it may be associated with the influence of a dietary or vitamine addition. The various investigations dealing with the effect of pregnancy on the growth of neoplasms may also be interpreted from the viewpoint of the chemical theory. From these investigations, we may see that the embryo contains a substance that stimulates the growth of neoplasms. The growing embryo, however, according to v. Graff (1559) and Slye (1560), needs this substance for its own growth, thereby inhibiting considerably the tumor growth.

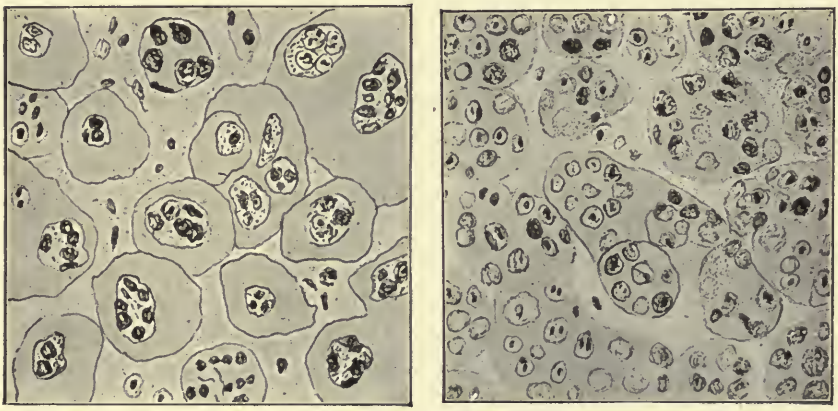
That the diet exerts some influence on tumor growth, was suspected by Ehrlich in his athreptic theory. This theory was tested experimentally by Jansen (1561) and Haaland (1562) who established the inhibiting influence of under-feeding. Similar experiments were also undertaken by Cramer and Pringle (1563), Rous (1564) and v. Jaworski (1565). The latter believed that under-feeding inhibits the growth of tumors. In his opinion, carcinomas were an exception since, under such conditions, they grew even more rapidly. The dietetic aspect of cancer research was stimulated by vitamine studies. Sweet, Corson-White and Saxon (1566), in their rat and mouse experiments, used the Osborne-Mendel diet and saw that the growth of tumors on a vitamine-poor diet was markedly arrested. In quick succession, there appeared papers on the same subject by Hopkins (1567), Centanni (1568), Rous (1569), Drummond (1570) (this work being very exhaustive), Benedict and Rahe (1571) and Sugiura and

Benedict (1572). Van Alstyne and Beebe (1573) believed that they had stimulated tumor growth by the addition of lactose to an artificial diet, but this was evidently due to a contamination of the lactose with vitamine B. In a similar manner, the author (l.c. 244, 245, and 246) showed in 1913 on chickens, that the chicken sarcoma of Rous grows much slower on a vitamine-poor diet than on one rich in vitamines, and these results were confirmed by Drummond (l.c. 247). Levin (1574), however, could not corroborate these findings and Fränkel and Fürer (1575) expressed themselves against this phase of cancer research. The few negative results, naturally, cannot have any effect on our conclusions in comparison with the conformity of the conclusions of a great number of investigators in this field. Undoubtedly, the restriction of the vitamines in the diet markedly inhibits the growth of neoplasms. This measure would have been of practical value, if it had not been evident that the tumor tissue shows a greater affinity for the vitamines than does the somatic tissue. This indicates that in order to obtain a complete success, the vitamines would have to be so far curtailed that the organism itself would die. That certain substances govern tumor growth, is evident from the paper by Rondoni (1576), who showed that the sarcoma tissue could favorably influence the growth of experimental tumors. The same conclusions were obvious from the work of Murphy (1577), who allowed rat tumors to grow in chicken embryo, it being impossible to do so in grown chickens. From this, it is evident that in rapidly growing embryonic tissue, a substance is present which stimulates the growth of neoplasms. That this view is justified, is clear from the work of Funk (1578), who showed that when the mouse chondroma of Ehrlich is implanted in the rat, the tumor tissue is resorbed in a few days. However, if the mouse tumor is fed to the rat in large quantities, then not only does the tumor develop but it may be transplanted in three generations of rats so treated. Here, in our opinion, it cannot be denied that the mouse chondroma contains a substance which acts specifically on the growth of a similar tumor. At the same time, the influence of the diet is shown very well. The induced tumor possessed, as the illustration shows, for the most part, the same histological structure.

The dietetic restriction for the purpose of treating cancer has also been used in practice. Bulkley (1579) used, for this purpose, a diet so chosen that it contained almost no animal protein. Since this

diet could be regarded for many reasons as pellagra-producing, we inquired as to the health of the patients and the duration of the therapy. Dr. Bulkley replied that a number of patients lived on the diet without showing any signs of pellagra. Copeman (1580) tried a similar therapy with a diet that was almost vitamine-free. At the time of his preliminary communication, his experiments had lasted from three to four months, and a detailed report was still to be made. His patients increased in weight. The dietetic treatment of cancer is at present not in favor with the medical profession.

We believe that it would be of value to cancer research if this disease were regarded more as a metabolic disturbance.



A B

FIG. 73. A, EHRLICH'S MOUSE CHONDROMA; B, THE SAME TUMOR GROWING IN A RAT AFTER TUMOR FEEDING

We shall now consider two problems in which the vitamines are of significance with greater certainty—the influence of the diet in the development of teeth, and the cause of cystic calculi and similar formations.

THE DEVELOPMENT OF TEETH

It was suspected by Kunert (1581) that the diet exerted some influence on the condition of the teeth. Durand (1582) saw a greater percentage of carious teeth in children who had been fed on condensed milk than in those fed on breast-milk. A carbohydrate-rich diet in particular, was held to be responsible for caries. Black and

McKay (1583) frequently observed a defective enamel in certain districts in the Rocky Mountains, where the diet was inadequate. Castilla (1584) often observed caries and defective enamel after colitis and other disturbances of the stomach and intestinal tract in children from 1½ to 4 years old. Considerable progress has been made in this field since the problem has been undertaken experimentally.

Miller and Gies (1585) studied the question of teeth formation in rats from the standpoint of the calcium and magnesium metabolism. M. Mellanby (1586) studied the condition of the teeth in young rachitic dogs. On diets that were poor in vitamine A, the following changes were visible:

1. Delayed falling out of the milk-teeth.
2. Delayed appearance of permanent teeth.
3. Disarrangement in the position of the teeth
4. Lack of or defects in the enamel.
5. Diminished calcium content.

In quickly growing teeth, the defects were even more pronounced. Vitamine A, in the form of cod liver oil or butter, exerted a very favorable influence. Zilva and Wells (1587) observed, in guinea pigs and monkeys on a scurvy-producing diet, a fibroid degeneration of the tooth pulp. These changes occur very early, often before all other scorbutic symptoms. The findings on scorbutic guinea pigs were confirmed by Percy R. Howe (1588), Robb, Medes, McClendon, Graham and Murphy (1588a) and developed further experimentally by P. R. Howe (1588b). The latter managed, by means of a partial lack of vitamine C, to keep the animals (guinea pigs and rabbits) alive for about one year, so that the results were more definite. The teeth were carious, poor in calcium and bent, with Pyorrhoea alveolaris. There was also observed a disease of the joints, resembling rheumatism or Arthritis deformans. Ballantyne (1589) found that 98 per cent of pregnant women show carious teeth. This may well be explained by the greater requirements for vitamines, calcium salts, etc. Sinclair (1590) rightly emphasized that precautions for the good condition of the teeth of the child must begin with the pregnant mother, who should receive the best possible nourishment. The effect of the diet on the teeth does not appear to be related to any one specific vitamine, but vitamine A, because

of its relationship to calcium metabolism seems to play the most important part. There is no doubt that the significance of the factors mentioned will soon receive greater attention in practice.

CALCULI

Osborne and Mendel (1591) found phosphatic calculi in the bladder in 857 rats kept on a diet poor in vitamine A. Padua (1592) saw a definite relationship between beriberi and calculi in the Philippines. Out of 58 cases of phosphatic calculi, 11 had beriberi; in 18 cases, under-nutrition was observed, but without any beriberi manifestations; 27 cases occurred in well-nourished patients. The phosphatic calculi were found mostly in children and young people; while urate calculi were observed later in life (after 50 years). Kirschner (1593) and Clemm (1594) observed that in Germany, on diets poor in fat, gall-stones are found more commonly. All these cases are associated with the indirect influence of the vitamines, presumably decreasing the resistance to infections, in which connection vitamine A is of special significance. For it is apparent that a local infection is the real cause of this calculus formation.

This concludes our data on the subject. We are well aware that despite the ten years of experimental and clinical research, most of the problems discussed by us have only been scratched on the surface. This is due largely to the fact that such slow progress has been made in the chemistry of the vitamines. In this connection, the frequent modifications of the nomenclature is not of the slightest value, and we must still await the investigator who, through tedious and patient work, will make the decisive step forward—the recognition of the exact nature of the vitamines.

Before closing, we shall add a few words touching upon the practical aspect of the vitamines. From what has already been said, it is evident that in a properly constituted diet there is no danger of an avitaminosis. In England and in the United States recently, there have appeared on the market a number of vitamine preparations whose purpose was to exploit the present popularity of the vitamines. There seems to be no doubt that most of these preparations cannot produce the effect claimed for them. Many investigators, among them Drummond (1595), have expressed themselves

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as being opposed to such preparations, maintaining that the natural foodstuffs may be used in the therapy and the prevention of avitaminoses. In the light of our present knowledge, this appears indeed to be the case. Still, we do not know how this matter will develop in the future, and the question can be studied only then when the vitamines will be obtained in the pure or highly concentrated state. It is possible that such pure products may manifest properties at present neither known nor even suspected. For example, on comparing the influence on rickets of vitamine A of butter and cod liver oil, it was found that only the latter can prevent the occurrence of the disease; one might therefore be tempted to regard the two substances as individual chemical entities. As a matter of fact, if it is true that cod liver oil is about 250 times richer in vitamine A than is butter, the above difference may perhaps be only one of concentration. Although most commercial products cannot withstand sharp scrutiny, it would be desirable not to decry the present concentration and purification experiments, since they may perhaps lead to interesting and important results.

GENERAL LITERATURE

In order to give the reader a broader survey of the field, there is appended a chronological list of some of the work done on vitamines during the last six years, insofar as the records were available and insofar as they will not be referred to again in the text. These references to the existing literature will enable the interested reader to make himself familiar with the views of other workers. Reports appearing from time to time in the daily newspapers have not been considered at all.

1914

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